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# INFLUENCE OF ANTI-RETICULAR CYTOTOXIC SERUM ON THE HEALING OF HUMAN FRACTURES

## I. ROENTGENOLOGICAL AND CLINICAL OBSERVATIONS*

MARTIN SCHNEIDER, HILBURN D. GILLIAM, C. M. POMERAT, ANDREW ORMSBY, G. W. N. EGGERS, AND WILLIAM H. AINSWORTH

This study is concerned with the effects on fracture healing of anti-reticular cytotoxic serum (ACS), or reticulo-endothelial immune serum (REIS), as it is also known. Our interest in this subject was stimulated by the Texas City disaster, of April 16, 1947, which afforded a group of fracture cases that came under observation simultaneously. During the following months, several additional fracture cases, observed and treated at the John Sealy Hospital, Galveston, Texas, were included in this series.

A complete review of the literature on ACS would be outside the scope of this report. An excellent review by Straus and his associates was published in 1946. In summary, Alexander A. Bogomolets conceived the idea that the various connective tissue cells of the body are more than inert supportive structures, and he broadened the concept to include in a "physiological system of the connective tissue" such diverse cellular elements as fibroblasts, lymphocytes, adventitial cells, osteoblasts, and the reticulo-endothelial cells, the latter particularly profuse in the spleen, thymus, liver, and bone marrow. He conceived of this system as performing trophic, plastic, protective, autoregulative, and mechanical functions within the body.

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*Read at the annual meeting of the Texas Radiological Society, January 17, 1948. From the Departments of Radiology, Orthopedics, and Anatomy (Tissue Culture Laboratory), and the Clinical Laboratories, The University of Texas Medical School and John Sealy Hospitals, Galveston, Texas. We are grateful to Eli Lilly & Co., Indianapolis, Ind., for financial aid and for the ACS used in this study. Received for publication September 20, 1949.

Bogomolets then followed up an hypothesis expounded in 1900 by Metchnikoff, that an organ-specific serum, toxic for a given tissue, would produce a stimulating effect on that tissue when administered in small amounts. In 1924, he succeeded in producing such a serum, specific for the physiological system of the connective tissue, by the injection into horses of tissue antigens rich in reticulo-endothelial elements, using human spleen and bone marrow from fresh cadavers.

Since then, research with ACS has been widespread in the USSR, but the first report in this country was presented before the Third International Cancer Congress in 1939, by A. A. Bogomolets. In 1941, there appeared a translated report concerning the effect of ACS on the healing of fractures, as reported by O. A. Bogomolets in the Russian literature. He claimed excellent results in experimental animals and humans, but warned that large doses had delayed fracture union.

Later reports have appeared in the Russian literature, but the only reported American investigations concerning the effect of ACS on fracture union are those by Straus and his associates. Maintaining strict criteria of progress they found statistically significant acceleration of fracture union in rabbits treated by stimulating doses of ACS as compared with controls and with animals given depressing doses of ACS. Their estimation of union was based on roentgen examinations and breaking-strength tests.

In human fracture cases, Thompson and his collaborators, including Straus, found a favorable influence on the rate of healing of fractures after use of ACS in stimulating dosage. Results in this group were evaluated by roentgen and clinical examinations, although the latter were not numerous enough for statistical analysis.

As an aftermath of the Texas City disaster, it was decided to proceed cautiously in testing the effect of ACS on the healing of fractures in man. We selected cases with diaphyseal fractures of the long bones only. Half the patients received ACS in "stimulating" doses, and the remainder served as controls, with normal rabbit serum injected. Each group was divided according to fracture type—simple or compound—and

then subdivided according to high or low blood alkaline phosphatase levels. Sex and race were not considered. The serological test for syphilis was negative in every case.

Eventually certain eliminations were made because of age. It was deemed unwise to include adolescents and children because of the strong tendency for rapid healing of fractures in that age group. Other cases were eliminated because of amputation necessitated by gangrene; because of fatal pulmonary infarction in one case; and because of internal fixation of some fractures dictated by clinical considerations. In the latter instances however, pertinent observations up to the time of operative intervention were included in our data. Five cases were added after the disaster, so that our series consists of 22 patients presenting 32 fractures (Table I).

Each group consists of 11 patients presenting 16 fractures. The control group includes 11 fractures of the lower extremities and 5 of the upper extremities. The ACS-treated group includes 13 fractures of the lower extremities and 3 of the upper ones (Table II). At the present time our series is obviously too small and our data too inadequate to attempt analyzing results in the sub-groups. As a matter of fact, the attrition in our original series has been such that our sub-groupings are no longer balanced. As a result, our control series of 16 fractures represents 6 simple and 10 compound fractures, while our treated series of 16 fractures represents 3 simple and 13 compound fractures. There are 7 comminuted fractures among the controls, as contrasted to 11 among the treated cases. This imbalance obviously militates heavily in favor of the controls, with respect to anticipated rates of healing. Our report compares all ACS-treated cases with all controls, regardless of the extent or type of the original injury, or of variations in the blood chemistries. Blood counts and chemistries have been performed rather extensively and will be reported at a later date.

Ages range from 18 to 63 years among the controls (average 32.5 years), and from 20 to 62 years in the treated group (average 34.6 years). Each group consists of 10 males and one female.

TABLE I

| Patient | Sex/Age | Bone Fractured (Series Number) | | Treatment: ACS | Treatment: Normal Rabbit Serum | Fracture Type: Simple | Fracture Type: Compound | Fracture Type: Comminuted |
|---|---|---|---|---|---|---|---|---|
| TB | F-20 | L. Femur | 1* | x | | x | | x |
| CC | M-23 | R. Tibia | 2 | | x | | x | |
| | | R. Fibula | 3 | | | | x | |
| | | L. Fibula | 4 | | | x | | x |
| MC | M-29 | R. Ulna | 5* | x | | x | | |
| JPD | M-37 | L. Tibia | 6 | x | | | x | |
| AND | M-21 | R. Tibia | 7 | x | | | x | x |
| | | L. Tibia | 8 | | | | x | x |
| | | L. Fibula | 9 | | | | x | x |
| RD | M-35 | L. Radius | 10 | | x | | x | x |
| JDF | M-37 | R. Femur | 11 | | x | x | | x |
| IF | M-39 | R. Ulna | 12 | | x | x | | |
| RAG | F-45 | R. Femur | 13 | | x | x | | x |
| APH | M-29 | R. Humerus | 14 | x | | | x | x |
| JJ | M-24 | R. Ulna | 15 | | x | | x | x |
| HBM | M-27 | R. Tibia | 16 | | x | | x | |
| | | R. Fibula | 17 | | | | x | |
| EM | M-20 | L. Tibia | 18* | x | | | x | |
| | | L. Fibula | 19* | | | | x | x |
| CM | M-20 | L. Femur | 20 | x | | | x | x |
| ELP | M-56 | L. Radius | 21 | x | | | x | x |
| RLP | M-26 | L. Humerus | 22 | | x | | x | |
| CS | M-18 | R. Humerus | 23 | | x | | x | x |
| JNT | M-62 | R. Tibia | 24 | x | | | x | |
| | | R. Fibula | 25 | | | | x | x |
| RW | M-21 | R. Tibia | 26 | | x | | x | |
| | | R. Fibula | 27 | | | | x | |
| WSW | M-63 | R. Tibia | 28 | | x | x | | x |
| | | R. Fibula | 29 | | | x | | |
| LCW | M-32 | L. Femur | 30 | x | | x | | |
| CY | M-55 | L. Tibia | 31 | x | | | x | x |
| | | L. Fibula | 32 | | | | x | x |

*Received only one course (three injections) of ACS.

It was our original hope to obtain radiographic studies simultaneously with the laboratory analyses at intervals of four weeks, but numerous considerations have precluded any such thorough follow-ups. Some of the patients moved from the area; fractures in other patients became clinically stable before reaching complete healing by roentgenographic standards and were therefore lost from observation; still other patients returned to their private physicians upon dismissal from the hospital. Consequently, our observations have been irregular.

Courses of ACS in stimulating doses were administered as soon as possible following injury, the longest delay in initiating treatment being twelve days in several of the Texas City patients. With three exceptions, each patient received three injections of ACS or normal rabbit serum at three day intervals, in doses of 0.05 cc., 0.10 cc., and 0.15 cc.; the entire course was repeated after four weeks. The three exceptions, noted in Tables I and II, are nevertheless included among the ACS-

TABLE II

| CONTROLS | | | | | ACS-TREATED CASES | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Bone No. | Number of Weeks at Which Each Healing Stage Was Observed | | | | Bone No. | Number of Weeks at Which Each Healing Stage Was Observed | | | |
| | 1 | 2 | 3 | 4 | | 1 | 2 | 3 | 4 |
| 2—Tibia | 18 | | | | 1—Femur† | | 7 | 12 | |
| 3—Fibula* | | | | | 5—Ulna† | | 5 | 9 | |
| 4—Fibula* | | | | | 6—Tibia | | 7 | 14 | |
| 10—Radius* | | | | | 7—Tibia | 6 | | 13 | |
| 11—Femur | 12 | 16 | 26 | | 8—Tibia | | 31 | | |
| 12—Ulna | 8 | 14 | | | 9—Fibula | 10 | | 31 | |
| 13—Femur | 18 | | | | 14—Humerus | | 10 | 24 | |
| 15—Ulna | 7 | | | | 18—Tibia† | 12 | 17 | | |
| 16—Tibia | 8 | 12 | 28 | | 19—Fibula† | 12 | 17 | | |
| 17—Fibula | 8 | 12 | 22 | 28 | 20—Femur | 6 | 10 | | |
| 22—Humerus | 8 | 13 | | | 21—Radius* | | | | |
| 23—Humerus | | 6 | 12 | | 24—Tibia‡ | | | | |
| 26—Tibia | 9 | 13 | | | 25—Fibula | | 16 | 33 | |
| 27—Fibula | 9 | 25 | | | 30—Femur | 10 | | | |
| 28—Tibia | 12 | 20 | 30 | | 31—Tibia | 18 | | | |
| 29—Fibula | | 30 | | | 32—Fibula | | 15 | 18 | |
| Average | 10.6 | 16.1 | 23.6 | | Average | 10.6 | 13.5 | 19.3 | |

*No healing during period of observation.
†Received only one course (three injections) of ACS.
‡Internal contact splint at 10 weeks because of non-union.

*Received only one course (three injections) of ACS.

treated group. We have graded healing according to the following arbitrary scale on the basis of serial roentgenograms:

0 no healing
1 minimal healing
2 moderate healing
3 advanced healing
4 complete union, with obliteration of fracture lines.

For purposes of comparison we graded and tabulated all examinations exclusive of the original roentgenograms made at the time of injury. Because of the irregular intervals at which these patients returned for follow-up studies, the only valid method of comparing healing in the ACS-treated and control groups seemed to be by tabulating the time intervals at which various stages of fracture union were observed in the individual cases.

The chart (Table II) summarizes those data, indicating for each fracture the time at which the various stages of healing were observed. This is not necessarily the time at which such healing first occurred. For example, fracture number 8, in the treated series, showed no healing at 14 weeks. It was not again examined until 32 weeks following injury, at which time Grade 2 healing was evident. We thus not only lost the observation of Grade 1 healing, but we do not know at what date Grade 2 healing first appeared.

If we now average the values obtained in the two groups, we find Grade 1 healing was observed at an identical average time in the two groups—10.6 weeks. For Grade 2 healing in the treated group, an average of 13.5 weeks was required as compared with 16.1 weeks for the controls; and in reaching Grade 3 healing, the ACS-treated cases averaged 19.2 weeks as compared with 23.6 weeks in the controls. It would appear, then, that Grade 2 and Grade 3 healing were slightly accelerated in the ACS-treated group.

By plotting the average rate of healing in the two groups against the time intervals involved, the graph shown in Figure 1 was derived on the basis of the above figures. Although our patients ranged from 18 to 63 years in age, and although six

different bones were involved, the distribution of these variables between the two groups is balanced.

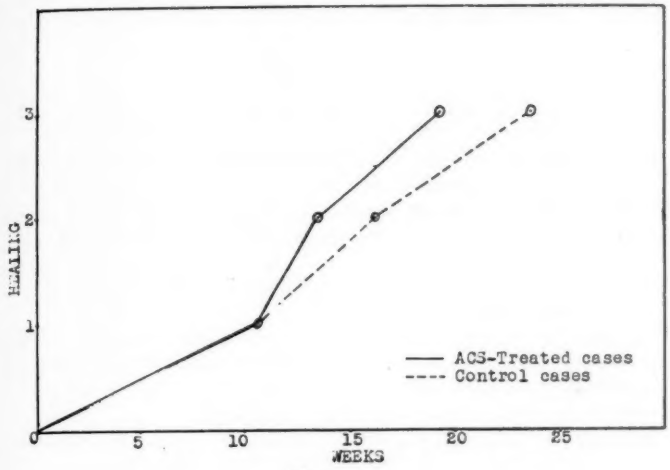


FIGURE 1

Graph based on Table II, showing number of weeks (average) at which each healing stage observed.

Clinical follow-up and observations of all cases were, of course, made routinely. Unfortunately these could not be performed in conjunction with the radiographic examinations in all cases, so that correlating trends were not sought. All patients who remained in this area eventually showed sufficient clinical healing to be discharged from medical care.

## SUMMARY AND CONCLUSIONS

Thirty-two fractures in 22 patients, most of them victims of the Texas City disaster, were observed roentgenologically and clinically with respect to rates of healing. Half of the group received stimulating doses of ACS, and the other half received equal quantities of normal rabbit serum.

There is wide variation in the types of original injury, with the ACS-treated group heavily overweighted with comminuted and compound fractures.

We do not feel justified in making a statistical analysis on the basis of these observations, but we believe that a tendency to faster healing may be suggested for the ACS-treated cases. Further conclusions must await more extensive observations.

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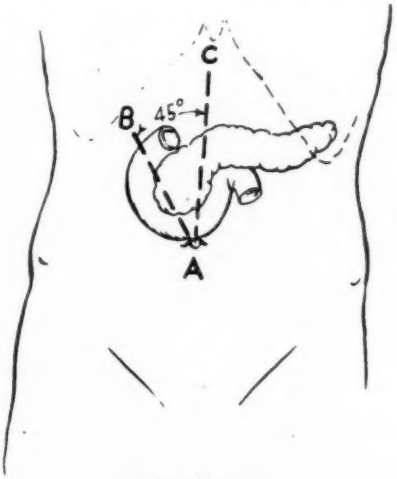
*This paper was published subsequent to the reading of the current report before the Texas Radiological Society, and is essentially the same as the personal communication from Straus, noted above.

# SURGICAL DISEASES OF THE PANCREAS WITH A NEW TECHNIQUE OF PRESENTATION*

EDGAR J. POTH

This paper represents a discourse presented to a medical meeting and to a class of medical students by using a novel teaching technique. The material is recorded on a wire which can be played back as often as desired and can be amplified to fill a hall of any size. The illustrations are shown by projection at the appropriate time and serve to clarify the subject matter being discussed.

Recordings such as these, together with the lantern slides, could easily be forwarded to remote areas and to small groups to which it would be impractical to send a speaker. The cost is relatively little. The master recording could be impressed



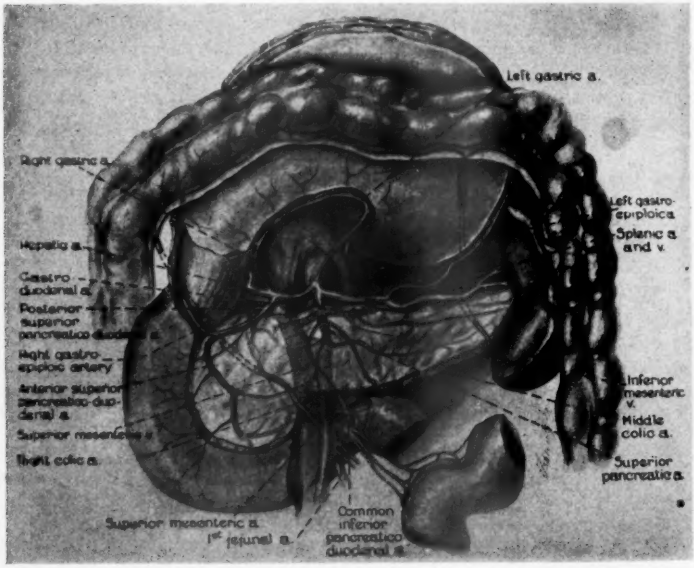

SLIDE NO. 1

Topographical, anterior projection of the location of the pancreas. Triangle BAC locates the most frequent area of palpable tenderness when the head of the pancreas is involved in inflammatory disease.

*From the Department of Surgery, The University of Texas Medical Branch, Galveston. Received for publication October 16, 1949.

upon a reel to fit the locally available sound reproduction equipment. A library of master recordings and lantern slides could represent a valuable asset and serve as another method of continuing graduate medical education.

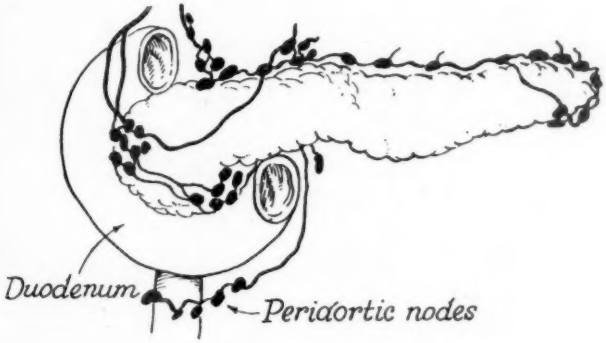
Today we shall discuss conditions which can be classified as surgical lesions of the pancreas. The term pancreas means "all flesh." The pancreas is a glandular structure deeply situated in the abdomen and lying transversely as indicated in this first slide. (Slide No. 1, please.) Notice that while the pancreas lies largely on the left side of the abdomen, it does extend across the midline and is outlined topographically on the anterior surface of the abdomen by a 45 degree angle designated by BAC. It is this area which is tender on palpation when the head of the pancreas is involved in inflammatory disease. Some of the anatomical relationships are shown in the next slide. (Slide No. 2, please.) The pancreas has a rich




Slide No. 2

Schematic representation of the anatomical relationships of the pancreas.

nerve supply from both sympathetic and parasympathetic fibers as well as a rich afferent network of sensory nerve endings. These latter are contained in the splanchnic plexus. This third slide demonstrates the rather rich lymphatic network about the pancreas which, together with the periaortic nodes, are the sites for drainage and extension of neoplastic lesions arising in the pancreas and in the distal end of the common bile duct. (Slide No. 3, please.)



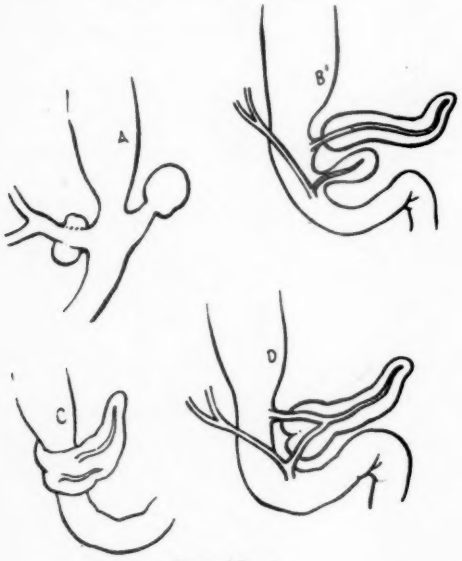

SLIDE No. 3

Lymphatic drainage of the pancreas.

Only during recent years has the pancreas been considered an important organ from the standpoint of the surgeon. Surgical conditions are now recognized which can be conveniently grouped under the headings of congenital, traumatic, inflammatory, and neoplastic lesions.

The next slide (Slide No. 4, please), shows a stage in the development of the pancreas with the organ budding out at two different points of the gastrointestinal tract giving rise to the ventral and dorsal pancreas. Herein we see a mechanism by which a congenital surgical lesion, the so-called annular pancreas, may develop. The abnormal development of the pancreas encircling the duodenum gives rise frequently to obstruction in the second part of the duodenum. Clinically, this lesion might really well simulate rather closely both obstruction at the pylorus of the stomach and also obstructions in the

distal portion of the duodenum brought about by the pressure of the superior mesenteric blood vessels across the duodenum just proximal to Treitz ligament. X-ray studies made of the patient in different positions, will in the one instance, show that the lesion is proximal to the duodenum proper, and, in the second instance, will show that it is in the second portion of the duodenum and that it is a constant lesion not alleviated by placing the patient in the head down position during the administration of the barium as would occur in the case of superior mesenteric ileus. (Lights, please.)




SLIDE NO. 4

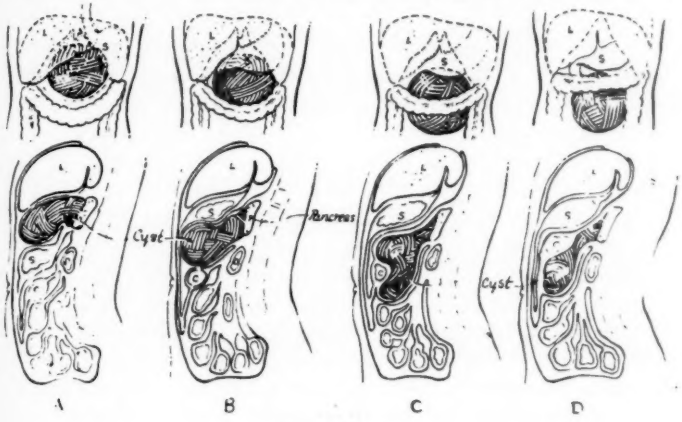
Embryologic development of the pancreas and the illustration of the mode of formation of "annular pancreas."

Fibrocystic disease of infancy probably is not a manifestation of congenital atresia of the ducts but is rather the late results of inflammation. Cystic anomalies may result from failure of proper fusion resulting in the lack of continuity of acinar tissue and duct systems.

The deep situation of the pancreas in the abdomen protects this organ from trauma, and when it does occur, especially where it crosses over the vertebral bodies, it is usually asso-

ciated with injury to other abdominal viscera. Trauma to the pancreas may give rise to pancreatitis which may be a serious complication especially in view of associated injuries in the other abdominal organs, including perforations in the gastro-intestinal tract, the secretions from which activate the pancreatic protein-splitting enzyme trypsinogen.

Not infrequently pancreatic cysts will develop following trauma; these are so called pseudo cysts because there is no true epithelial lining. They may present anteriorly in the various manners demonstrated in the following slide (Slide No. 5,

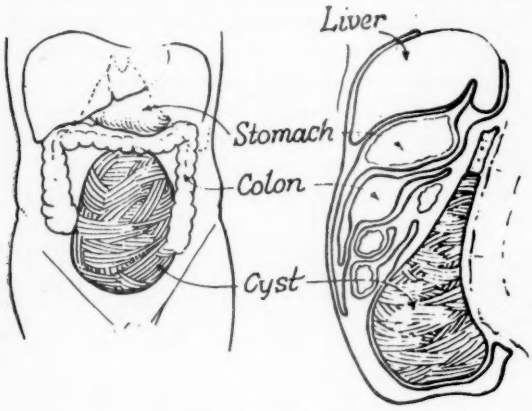



SLIDE NO. 5

Illustration of the sites of presentation of cysts of the pancreas.

please), and give rise to some rather interesting differential diagnostic problems. As you see in this slide, the cyst might present itself above the stomach coming anteriorly through the gastrohepatic ligament displacing the stomach downward. On the other hand, it might present itself as in the second diagram, coming through the lesser peritoneal sac to push the stomach upwards and the transverse colon downwards, to present through the gastrocolic omentum. Or, the presentation might be through the gastrocolic omentum primarily, pushing the transverse mesocolon downward. The lesion might dissect between the leaves of the transverse mesocolon to present both above and below the colon. Again, the displacement and presentation might be entirely below both the stomach and the

colon with an upward displacement of both these organs. As is indicated in this next slide (Slide No. 6, please), the cyst can




SLIDE NO. 6

Demonstration of the mechanism by which a cyst of the pancreas may simulate an ovarian cyst.

dissect entirely retroperitoneally and present itself first in the pelvis, where it would most likely be diagnosed as a cyst to an ovary in the female. These various regions of presentation of cysts of the pancreas must be appreciated if one is to give a proper interpretation of the findings of careful X-ray examination. Definitive diagnosis usually will be made at exploration. Recently a rare traumatic cyst of the liver was found. The preoperative diagnosis was pseudo-cyst of the pancreas. Treatment of pancreatic cysts will be discussed later. (Lights, please.)

### *Acute Pancreatitis*

One of our most important lesions and one which offers considerable difficulty in diagnosis is acute pancreatitis. Acute pancreatitis is not at all a rare disease. Unfortunately it is diagnosed relatively infrequently. Final diagnosis depends to a large degree on laboratory findings. Acute pancreatitis is difficult to classify into simple pathologic conditions; it is

neither an acute infectious or inflammatory process, nor a clearly necrotizing or hemorrhagic lesion. It is usually a combination of all of these processes at the same time. It is a biologic entity in itself, different from the pathology of other organs because of the peculiar physiologic and biochemical properties of this organ. Acute pancreatitis is a comprehensive name for all the acute disorders of the pancreas. Such terms as acute pancreatic necrosis, hemorrhagic pancreatitis, edema of the pancreas, interstitial pancreatitis, transient pancreatitis, fat necrosis of the pancreas, and so forth are used in accordance with the pathology or the clinical course.

For the purpose of clinical discussion, it is best to divide pancreatitis into the mild and severe forms. The gross pathology in the mild cases is usually a glassy edema of the pancreas and of the peripancreatic tissue extending into the mesocolon or into the mesentery with varying amounts of slightly bile colored or slightly hemorrhagic peritoneal exudate. A moderate amount of fat necrosis is often seen around the pancreas in the root of the mesentery or in the omentum. In the severe cases, large amounts of moderately hemorrhagic, less often, bile colored peritoneal exudate are found. Fat necroses are often seen throughout the abdomen. The pancreas is swollen and either studded with fat necroses or the area of the pancreas is transformed into a bluish-black mass. On sectioning this mass, one sees that the pancreas is much less hemorrhagic than it appears from the outside, and that only part of the pancreas is hemorrhagic or that the hemorrhagic mass consists only of the peripancreatic tissue. In other cases the pancreas is completely necrotic and hemorrhagic. According to the pathology in each case, the condition is called either hemorrhagic pancreatitis, hemorrhagic necrosis, apoplexy of the pancreas, pancreatic necrosis, or fat necrosis of the pancreas. The question of whether these two types are entities or only different stages of the same process has not yet been entirely settled. It seems most likely that they are different stages of the same process because it is not unusual to see a case showing typical edema of the pancreas at operation and then show a necrosis of the pancreas at necropsy a few days later. Furthermore, the same pathologic mechanism may be demonstrated in all forms of

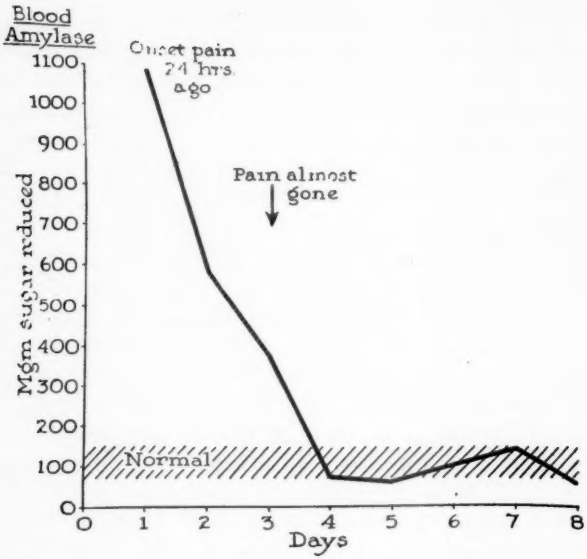
acute pancreatitis and sometimes a mild form of the disease turns into a more severe one. There are instances in which necrosis of the pancreas exists from the beginning of the disease. The differentiation between mild and severe forms is of great clinical importance. But, the clinical symptoms and pathologic changes do not always run parallel. Not infrequently, there will be only minor changes of the pancreas in the presence of an unusually severe disease clinically. While there are controversial views on certain points, the etiology of acute pancreatitis is fairly well understood. It is generally agreed that acute pancreatitis is due to the action of activated pancreatic juice upon the pancreas itself. The conversion of trypsinogen into trypsin by duodenal juice, bile, bacteria, and occasionally the spontaneous activation of the trypsinogen accounts for the digestion of the protein of the pancreas itself.

Lipase likewise plays a major role. The unusual features of acute pancreatitis is explained by the action of these enzymes on the glandular tissue. This activity is indeed remarkable and can be demonstrated in the experimental animal by dividing the pancreatic duct and then giving the animal pilocarpine, resulting in a marked elevation of the temperature of the surrounding tissues to such a degree that it is uncomfortable to place ones hand in the abdomen of the sacrificed experimental animal. Fat necrosis may extend not only into all the abdominal viscera, but it may extend through the entire thickness of the abdominal wall. The edema fluid is rapidly absorbed into the general circulation, and accounts for the increased blood enzyme values which are found experimentally as early as five or ten minutes after damage to the gland. In the presence of pancreatic edema, temporary occlusion of the pancreatic artery might cause necrosis of the pancreas.

In 1901 Opie postulated that acute pancreatitis was the result of regurgitation of infected bile into the pancreas following blockage of the ampulla of Vater in the presence of a common channel; *i.e.*, by the pancreatic duct joining the common bile duct proximal to the ampulla. This blockage might be due either to a stone, a tumor, or spasm of the sphincter of the ampulla. Numerous anatomical studies have demonstrated that approximately 20% of the biliary and pancreatic ducts

form a common channel. In 60 to 80 per cent of cases of acute pancreatitis, there is associated pathologic changes in the biliary tract. By studies on the cat it has been shown that the injection of sterile bile into the pancreas will cause the development of edema. Hemorrhagic necrosis occurs if the bile contains bacteria. The clinical picture of acute pancreatitis is not characteristic either in complaint, history, or physical findings.

Some features however prevail and are suggestive of pancreatitis. The disease is extremely rare in children but has been reported in all other age groups. Some statistics show a greater incidence in men; some in women. Frequently the patient is obese, but this is not necessarily the case. Some are alcoholic; many are not. A history of epigastric pain, interpreted as previous gallbladder attacks, is present in 50 per cent of the cases. Ordinarily, the patients are not jaundiced, although there might be a slight increase of the icterus index. Characteristic findings are those of severe pain in the epigastrium radiating to the left and to the back. The pain may follow an episode of a few days of indigestion. On the other hand, it might come on suddenly, not rarely following a heavy meal or during excessive drinking. A tentative diagnosis of acute pancreatitis should be made from a classical description of excruciating pain which is not relieved by morphine. It might be difficult to differentiate this pain from that arising from other abdominal or renal conditions. However, if it is anterior and extends to the left of the midline, it should arouse the suspicion of acute inflammation of the pancreas. Then one should resort immediately to laboratory tests, especially determination of the blood amylase. The characteristic elevation of serum amylase with its return to normal even during continuation of the disease is illustrated in the following slide. (Slide No. 7, please.) Urinalysis frequently shows albumin and various types of casts, especially in severe cases. Glycosuria is seen in 5 to 20 per cent of the severe cases; rarely in the mild forms. Traces of bilirubin are found occasionally in the urine and increased urinary urobilinogen is seen rather frequently. The blood count frequently shows white cells to be around 20,000 with polymorphonuclears predominating.




SLIDE No. 7

Characteristic blood amylase levels in acute pancreatitis.

The blood non-protein nitrogen and urea are at the upper levels of normal and as a rule are markedly elevated in severe cases. There is frequently a slight elevation of the icterus index. Fasting blood sugar is usually above normal and may be markedly elevated in the severe forms. There may be a decrease of serum calcium in proportion to the amount of fat necrosis. Amylase is rapidly excreted into the urine and may be found to be elevated in the urine after the blood serum level has dropped to an essentially normal concentration. It is not an unusual finding to observe that the most severe cases of acute pancreatitis will not show the greatest elevation of the serum amylase. It is felt that this might be due to the fact that the pancreas has been destroyed. (Lights, please.) Clinically these cases may be divided into three general types on physical examination. One, those with severe toxic reaction which present a shock-like picture. They have marked tenderness and some

rigidity all over the abdomen, but more pronounced in the epigastric region. The pulse rate is high, and there might be a mottled cynosis of the abdomen and limbs. These patients look very much as if they were suffering from an acute vascular accident such as coronary occlusion or embolism of the mesenteric vessels. Or, they may appear to have some overwhelming infection. Too, cases with predominately peritoneal symptoms of marked tenderness and rigidity make the differentiation from perforated peptic ulcer difficult. The rigidity rarely attains the board-like quality of a perforation. This group comprises mild and severe cases and early separation of the two forms is often not possible. Three, the third clinical form is characterized by intestinal paresis as the predominant finding. These cases show very little rigidity, but some tenderness and a gradually increasing distension in the abdomen. They are usually light cases, and simulate renal or intestinal colic.

Formerly, acute pancreatitis was considered a surgical emergency and either incision of the pancreas with drainage or, more recently, drainage of the gallbladder and common duct was performed. Mortality from these procedures ranged around 50 per cent. This high mortality has brought about a gradual change to a more conservative type of treatment and has resulted in considerable improvement. In cases in which the diagnosis is doubtful, exploration probably causes no harm in the mild cases provided the pancreas is not manipulated. The operation should be terminated immediately when the condition is recognized, possibly with drainage to the gallbladder.

Some surgeons still contend that the more severe cases should be explored and drained. It is doubtful, however, that this course will save as many patients as a proper conservative management. An important disadvantage of surgery in severe cases is the rather frequent incidence of wound disruption, formation of pancreatic fistulas, or of complicated wound infection with a much prolonged illness. The aim of conservative therapy is to diminish the secretory activity of the pancreas, and to relieve the spasm of the sphincter of Oddi. This is best accomplished by avoiding any stimulation of the pancreas, by inhibition of pancreatic secretion, and by release of the tone of the sphincter of Oddi. Anything that stimulates the vagus

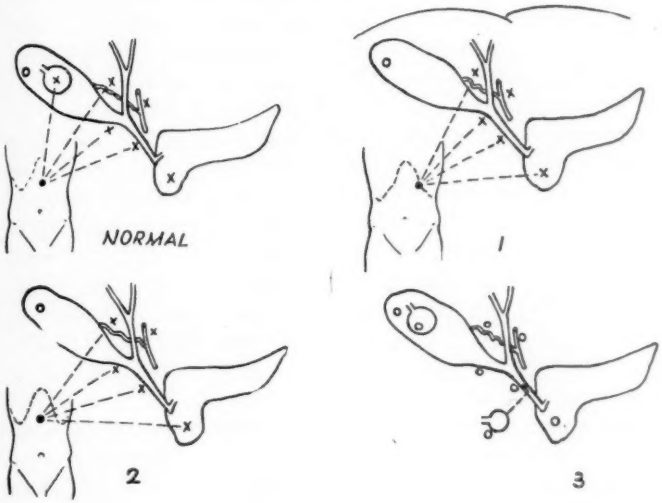
nerve will stimulate the pancreas. Therefore, prostigmine or morphine should be excluded. The vagotonic action of morphine might conceivably be the reason there is insignificant pain relief after morphine administered in acute pancreatitis.

Hyperglycemia has a pronounced stimulating effect by the cholinergic mechanisms and hypoglycemia has a marked vagotonic action. Parenteral glucose administeration as well as the regulation of the blood sugar by insulin must be handled extremely carefully. The patient should receive nothing by mouth and continuous gastric suction should be instituted to keep the stomach empty and to prevent hydrochloric acid from entering the duodenum. Ephedrine and atropine should be given in repeated doses to inhibit pancreatic secretion. Demerol with its atropine-like action is probably the analgesic of choice, and should be given instead of morphine. Nitroglycerine and other antispasmotics might be of some value.

Intravenous fluids should consist mainly of saline solution and blood plasma. Glucose should be given only with careful control of the blood sugar and should be combined with atropine. Gage and de Takats have reported excellent results following splanchnic block. Gage has repeatedly stated that this is the only proper treatment for acute pancreatitis. As illustrated in the next slide (Slide No. 8, please), it is evident that bilateral splanchnicetomy is required to relieve pain. It is thought the beneficial effects may be due to a decrease of pancreatic secretion and release of the arteriospasm following interruption of these nerves to the pancreas, and also to the inhibition of the sphincter of Oddi. On the other hand, in cases of severe acute pancreatitis with necrosis it is felt that a lethal dose of histamine-like decomposition products has already been absorbed before any therapeutic measure can be instituted. (Lights, please.)

Following recovery from acute pancreatitis, it is necessary to carry out certain measures to reduce the frequency of future attacks. The patient should abstain from heavy, rich food and any substance which might cause irritation to the biliary tract. In the event there is disease of the biliary tract, cholecystectomy should be performed to remove stones and to irradicate a possible source of future mechanical or physiological blockage at

the ampulla of Vater and consequent regurgitation of bile into the pancreatic ducts.




SLIDE NO. 8

*Pancreatic Pain*

1. After right splanchnic resection stimulation of gall bladder, biliary ducts, and pancreas gives pain referred to epigastrium to left of the midline.
2. After left splanchnic resection stimulation of gall bladder, biliary ducts and pancreas gives pain referred to epigastrium to right of the midline.
3. After bilateral splanchnic resection stimulation of gall bladder, biliary ducts and pancreas fails to give rise to pain.

## *Chronic Relapsing Pancreatitis*

In 1946, Comfort, Gamble and Bagenstoss of the Mayo Clinic clearly differentiated a chronic relapsing pancreatitis from other pancreatic disease. This report was followed 18 months later by one from the Cleveland Clinic by Bartunek and Collins. The condition is characterized by recurring attacks of pain in the upper part of the abdomen, and at the same time is associated with disturbances in function of the acinar and islet

cells of the pancreas and certain sequellae. These include principally calcification and more rarely diabetes, steatorrhea, and creatorrhea. The disturbances of the secretory function are ordinarily mild and transient with the first relapses of this chronic disease, tend to become more prominent with each attack, and may become permanent. The cause of this disease has not been definitely established. Certain pathologic changes are found at operation or necropsy and vary from edema, inflammation, hemorrhagic necrosis, and abscess in the acute phase to atrophy, fibrosis, cyst formation, calcification, and stones in the late stages. The damage tends to persist and progress between the clinical attacks. The essential pathological progress seems to be either repeated attacks of acute interstitial pancreatitis, repeated sublethal episodes of acute hemorrhagic pancreatitis, or a combination of the two. Apparently calcifications and pseudo-cyst formation occur secondary to the inflammatory process. Interstitial fibrosis, necrosis, and atrophy are constant chronic changes. In many patients with chronic relapsing pancreatitis, there is associated disease of the gallbladder and biliary tract, or duodenal ulcer. But, although in some cases the relationship may be most suggestive, the involvement of the pancreas alone occurs frequently enough to conclude that it represents a clinical entity.

The frequency with which the diagnosis can be made is directly in proportion to the suspicion aroused. Biliary colic, cholecystitis, perforating or penetrating duodenal ulcer, aortic aneurysm, nephrosis, intermittent intestinal obstruction, tabetic crisis, and heart disease are usually first considered and must be ruled out. Carcinoma of the pancreas or ampulla of Vater may cause pancreatitis which at first resembles primary chronic pancreatitis. But, carcinoma is continuous and progressive. Retroperitoneal tumors, and non-tropical sprue must be excluded, the latter when steatorrhea is present. Pain is the most characteristic symptom. It is usually steady, severe, and prolonged. The onset is usually gradual, increasing to a plateau and then declining gradually. Analgesics are usually required to control the pain. Again, morphine should not be given, but demerol is preferred as in the instance of acute pancreatitis. A cycle of pain may require hours or days to develop and subside,

and then the patient may be free from pain from a matter of hours to years before another attack develops.

Location of the pain in order of frequency is epigastric, right upper quadrant, and left upper quadrant. It may occur as generalized abdominal, right lower quadrant, or periumbilical, and it may have the characteristic distribution of cholecystic disease. The extension of pain is characteristically to the back, and the lower thoracic and upper lumbar region, but it may extend anywhere in the abdomen and chest. The seizures of pain may be accompanied by nausea, vomiting, diarrhea, constipation, chills, fever, shock or jaundice. These symptoms are seldom severe. Steatorrhea and creatorrhea are late symptoms because the pancreas, like the liver, has a tremendous functional reserve and advanced destruction is necessary before failure of external secretion results.

The age distribution is from late childhood to advanced years, but occurs most frequently in middle life. There is no sex predilection, and overindulgence in alcohol and food is believed by some patients to initiate an attack. Alcoholism is not unusual, but it may be difficult to decide whether alcohol was used excessively to relieve pain, or whether its abuse antedated the pain. Not infrequently, alcoholic patients will abstain from alcohol after the pancreatic pain has been relieved surgically.

Physical findings are primarily of importance in helping to rule out other causes of upper abdominal pain. The pancreas lies so deep in the abdomen it is seldom palpable either in health or disease. When a mass arising from the pancreas is palpable, it is usually a cyst secondary to damage from previous attacks. During the height of a severe episode, there may be epigastric tenderness of spasm, more often on the left than the right, moderate jaundice, slight fever, or mild shock. Between attacks there might be some epigastric tenderness and evidence of weight loss but little more. The laboratory diagnostic aids are limited to demonstrating evidence of infection and pancreatic insufficiency during a relapse, or pancreatic insufficiency and stone formation when the patient is investigated between the painful episodes. Leucocytosis is not an important finding, but may occur. Urinalysis contributes nothing except when

glycosuria is present. This finding is then demonstrated to be due to pancreatic insufficiency by blood sugar determinations and a glucose tolerance curve. Transient diabetes is not unusual during painful relapses of the disease, but only rarely does it persist after the episode subsides. Urinary diastase may be elevated during the attack, and rarely between episodes. Stool examinations offer little. There may be increased fat but this finding occurs also in patients with pancreatic insufficiency due to malignancy. The values for activity of serum amylase and lipase are the most easily demonstrated pancreatic enzymes in the blood, and elevation above normal occurs with sufficient frequency during painful relapses to warrant determination in any case of obscure upper abdominal pain. The values are only rarely elevated between the attacks. Tests of hepatic sufficiency do not contribute to the diagnosis of chronic relapsing pancreatitis. Roentgenograms of the pancreatic area should always be done. Calcifications in the pancreas can be demonstrated in 50 per cent of patients sometime during the course of the disease. Often it is these findings which direct the surgeon to suspect the condition. The presence of a pancreatic cyst is usually demonstrated by external pressure on the barium-filled stomach, duodenum, jejunum, or the transverse colon as previously discussed. Invasion or obstruction of these viscera indicate malignancy of the pancreas rather than a benign cyst associated with chronic relapsing pancreatitis.

When no pancreatic insufficiency or calcification exists, the laboratory can give little to aid the clinician in establishing the diagnosis. If the patient's pain is severe, and relapses are frequent, surgical exploration to establish the diagnosis and correct any concurrent disease of the biliary tract is not only justified but advisable. The definitive diagnosis frequently rests on the findings at exploration. The pancreas may be hard and firm, or hard and nodular like carcinoma, and sometimes has a diffuse inflammatory edema. Cysts may be recognized and dealt with. It is not unusual for these patients to have been subjected to multiple exploratory laparotomies with the removal of non-symptomatic gallbladders and appendices before the correct diagnosis is made. The surgeon must always

focus his attention on this possibility during exploration because of repeated upper abdominal pain.

The treatment is primarily surgical. Medical treatment of chronic relapsing pancreatitis is limited to symptomatic palliation and replacement therapy for insufficiencies of pancreatic secretions both internal and external. Since there is evidence that the healthy pancreas elaborates fat metabolites related to the prevention of fatty degeneration of the liver, choline chloride should be administered. The diet best tolerated is similar to that used in the treatment of irritable colon and eliminates fried food, raw fruits and vegetables, and spices. The relative amounts of carbohydrate, protein and fat in the diet will depend upon the pancreatic deficiency and will vary, not only between patients but also in the individual as the disease progresses. Partially digested proteins are beneficial when the patient is malnourished, and there must be an adequate vitamin-mineral intake. Alcohol should be prohibited. Surgery at present offers the most to these patients. Not only is the diagnosis and degree of pancreatic involvement established by exploratory laparotomy, but at the same time certain complications can be dealt with. Correction of biliary disease, drainage of pancreatic cysts or abscesses, removal of calculi from the large pancreatic ducts, anastomosis of the gallbladder to the intestines, or drainage of the common bile duct are often indicated.

Partial or total pancretectomy has been employed to relieve intractable pain. However, before undertaking such an extensive procedure, vagotomy and resection of the splanchnic nerves should be done in an attempt to relieve the severe pain which is the most disabling part of this disease. If the attacks are of infrequent occurrence, and are of short duration, the administration of demerol and atropine may be sufficient when combined with a proper dietary management.

Chronic, relapsing pancreatitis is a clinical entity whose chief disabling symptom is severe upper abdominal pain. These patients are frequently diagnosed as psychoneurotics. A diagnosis of psychoneurosis should never be made by a surgeon. Such diagnoses should be made only by a psychiatrist, and, after it is made, the surgeon should always look upon it with suspicion.

*Tumors of the Pancreas*

Tumors of the pancreas have become important surgical lesions within the past few years. These tumors may be either benign or malignant, cystic or solid. Among the cystic tumors, we have several groups. The psuedo-cyst resulting from trauma or spontaneous necrosis of the pancreas have already been discussed. Retention cysts result from obstruction of the duct and are characterized by having an epithelial lining. The congenital cysts are usually multiple and are frequently associated with cystic anomalies of other viscera, and ordinarily result from imperfect fusion of the several embryonal elements, resulting in lack of continuity of the acinar and duct systems. Finally there are the proliferative cysts or the cyst adenomas which arise from glandular epithelium and are true tumors. These proliferative elements may undergo malignant degeneration and consequently they represent a very important group of cysts from the standpoint of treatment and prognosis.

A limited number of surgical attacks are available for the management of pancreatic cysts. Each method has some merit and certain limitations. A careful appraisal of the relevant features of individual case will suggest the proper procedure in each instance. Marsupilization or external drainage has been the most popular method of treatment in the past. That this procedure is still widely employed indicated that it is effective in many instances. But cumulative experience has shown that marsupilization is followed frequently by recurrent cyst formation or by persistent external fistulas. Drainage of these fistulous openings may persist for many years and the escaping pancreatic ferments can cause painful erosion about the stoma. Secondary infection of the sinus and recurring cysts may contribute to the intractability of the drainage. Irridiation or chemical cauterization may eliminate some of these fistulas. Marsupilization and external drainage should properly be confined to the treatment of pseudo-cysts before they have acquired thick, rigid walls or epithelial lining. Where the cyst has developed an epithelial lining, complete local excision should be practiced. This is particularly true in cases of proliferative cysts because of their propensity for malignant degeneration. In any instance, the dissection must be carried out

very carefully and patiently. There may be extreme hazards from the standpoint of hemorrhage or damage to adjacent viscera. Because of the likelihood of entering adjacent bowel, it is important that these patients be treated with sulfasuxidine and sulfathalidine before operation is undertaken. Occasionally the pancreas is so involved in the growth that a partial pancreatectomy is more feasible than local enucleation. Occasionally a small inflammatory cyst in the head of the pancreas will simulate so closely carcinoma, that a pancreatoduodenectomy will be performed. For other than proliferative cysts of the pancreas, internal drainage may occasionally be the most desirable procedure. In this event, drainage is best secured by anastomosing a loop of jejunum to the cyst using the Roux-Y technique.

Adenomata of the pancreas with cells closely allied to the beta cells of the normal islets of Langerhans with the production of hypoglycemia have attracted considerable attention during recent years chiefly because the symptoms of hypoglycemia are so dramatic. Spontaneous hypoglycemia from many causes is relatively common; but that caused by true hyperinsulinism is quite rare. Hepatic insufficiency may be a direct cause of hypoglycemia; deficiency in secretion of the anterior lobe of the pituitary body may likewise result in hypoglycemia.

Removal of the anterior pituitary produces a hypoglycemia which can be alleviated by pancreatectomy. Hypoglycemia may be a prominent feature in adrenal cortex deficiency. In the absence of a tumor in the pancreas, hypoglycemia has been attributed to a diffuse hyperplasia of the islets, but it appears more logical to explain the hypoglycemia in such cases by a mechanism similar to that just described; that is, pituitary and adrenal hypofunction, particularly because microscopic proof of actual hyperplasia is only rarely demonstrable. Hyperinsulinism may result also from carcinoma of the islets. The recovery following simple excision of an adenoma of the beta cells is indeed dramatic. Immediately following excision of such an adenoma the patient frequently will have a transient diabetes which will clear in the course of a few weeks. If no adenoma is found at the time of operation, and one is quite certain that the pituitary and adrenals are not involved, then

a subtotal resection of the pancreas might be undertaken. Occasionally there is more than one adenoma and a careful search of the pancreas must be made.

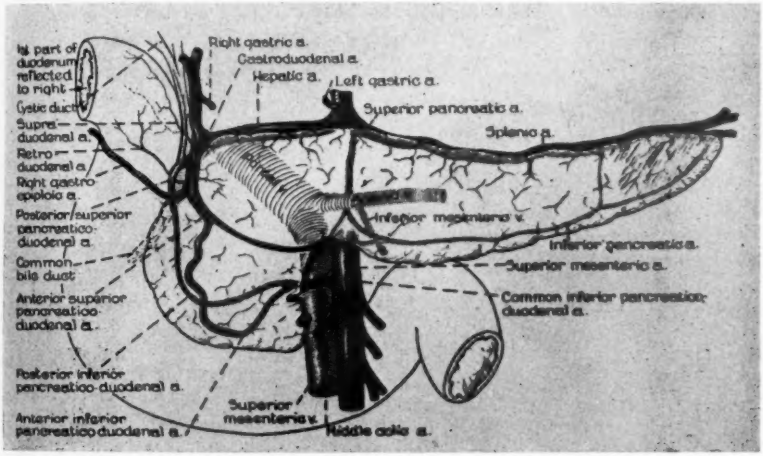
### *Carcinoma of the Pancreas*

There has been a great deal of interest lately in resections of the head of the pancreas for carcinoma in this general area. This includes resection for carcinoma of the ampulla, carcinoma of the head of the pancreas, and carcinoma of the lower end of the common bile duct, as well as occasional carcinoma arising in the mucosa of the second portion of the duodenum. Of these malignancies, carcinoma of the head of the pancreas is somewhat more frequent. If the tissues are examined during the early stages of the disease, carcinoma of the head of the pancreas can usually be demonstrated to have arisen from the pancreatic ducts. Later the origin is less definite because of the spread of the lesion. Since only one to two per cent of all malignancies arise in the pancreatoduodenal area, the total experience in the surgical management of these lesions is somewhat limited. A sufficient operation for malignancies in this area calls for a block resection of the head of the pancreas, lower end of the common duct, pylorus of the stomach, and the duodenum and frequently portions of the jejunum. This is an extensive operative procedure and should not be undertaken unless there is some hope of cure, especially since added experience has shown that once there has been extension from the original site life expectancy is rather attenuated. Whenever there is persistent, progressive jaundice, one must suspect malignancy in the area. While classically it is considered that carcinoma of the head of the pancreas or the ampullary region gives rise to a painless type of jaundice, this is not necessarily true because mechanical obstruction of the pancreatic ducts with dilatation of these structures may give rise to severe pain, especially if there is an accompanying pancreatitis. Approximately 25 to 30 per cent of the malignant lesions in this region can be resected sufficiently widely to give some hope of cure.

The prognosis is most favorable in the cases of carcinoma of the ampulla, while those of the pancreas and biliary ducts and

duodenum *per se* give a much less favorable outlook. During abdominal exploration, careful search should be made for evidence of metastasis. Biopses taken directly from the pancreas are usually not very helpful because one can either make the diagnosis from gross examination or the lesion will be sufficiently deep that it will not be obtained in the biopsy specimen.

A biopsy of the liver should always be taken. Because, even in the absence of any demonstrable evidence of extension of the lesion and negative palpation of the liver, microscopic metastases may be demonstrated on frozen section. In which event, of course, no extensive operative procedure would be carried out. This slide (Slide No. 9, please), shows the blood supply

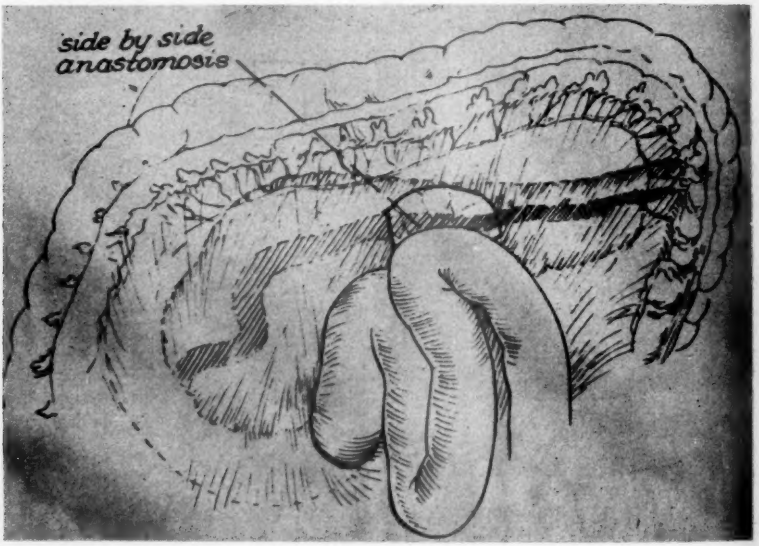



SLIDE NO. 9

A schematic representation of the main blood supply to the pancreas.

in the region of the pancreas rather diagrammatically and, if one will recall slide number three, on the location of the lymph nodes, it becomes obvious that the nodes along the hepatic artery and those at the superior aspect of the pancreas and below the common duct are likely to be involved and should be biopsied. Direct invasion and local spread can be demonstrated in the region of the inferior vena cava. Unless the area can

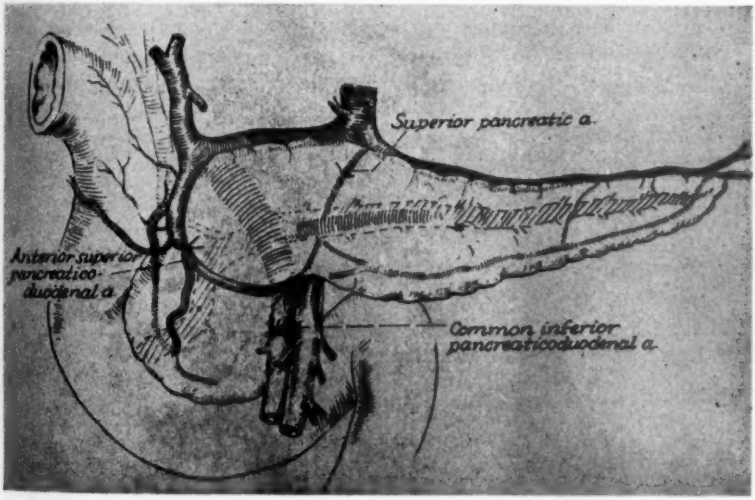
be elevated beyond the malignancy, resection should not be contemplated. Involvement of the superior mesenteric vessels can be determined by division of the gastrocolic omentum and incision of the peritoneum on the interior border of the pancreas. Similarly, the portal vein can be identified by division of the gastroduodenal artery and displacement of the common duct downward, permitting digital examination of the posterior surface of the head of the pancreas, displacing the portal vein backward. If the portal vein is involved, resection is contraindicated. Distant metastasis, local spread, with direct invasion beyond the limit of resection, involvement of the superior mesenteric vessels, and involvement of the portal vein are four findings which definitely contraindicate operation with the hope of doing a block dissection and perfecting a cure.




SLIDE NO. 10

**Indication of the site for anastomosing the pancreatic duct to the jejunum in inoperable carcinoma of the head of the pancreas. This procedure relieves pain and allows nutritional improvement by returning the digestive ferments to the bowel.**

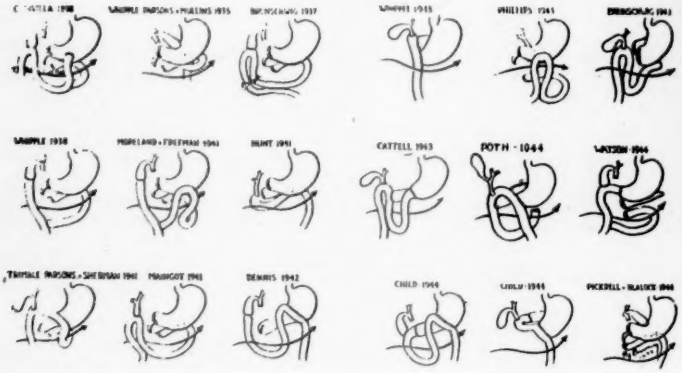
The life expectancy of a patient with carcinoma of the head of the pancreas is such that approximately 75% of the patients will die within nine months after the onset of their obstructive jaundice. There are, however, certain palliative procedures which are worthwhile. The relief of the obstructive jaundice, and the accompanying pruritus is a welcome relief to the patient. Simple relief of the obstructive jaundice, however, does not greatly improve the nutritional state of the patient if there is an accompanying block of the pancreatic ducts. One should consider the following palliative procedures for inoperable carcinoma of the head of the pancreas. Cholecystojejunostomy, by the Roux-Y technique; pancreatojejunostomy, as illustrated in this slide (Slide No. 10, please), by which a side-to-side anastomosis is made between the dilated pancreatic duct and a loop of jejunum preferably in the Y created above. If the pancreatic duct is very large it can be opened and a side-to-side suture be performed between the duct and the jejunum. On the other hand, if the patient's condition is poor




SLIDE No. 11

Shows the points at which the arteries to the pancreas may be ligated in inoperable cancer of the pancreas.

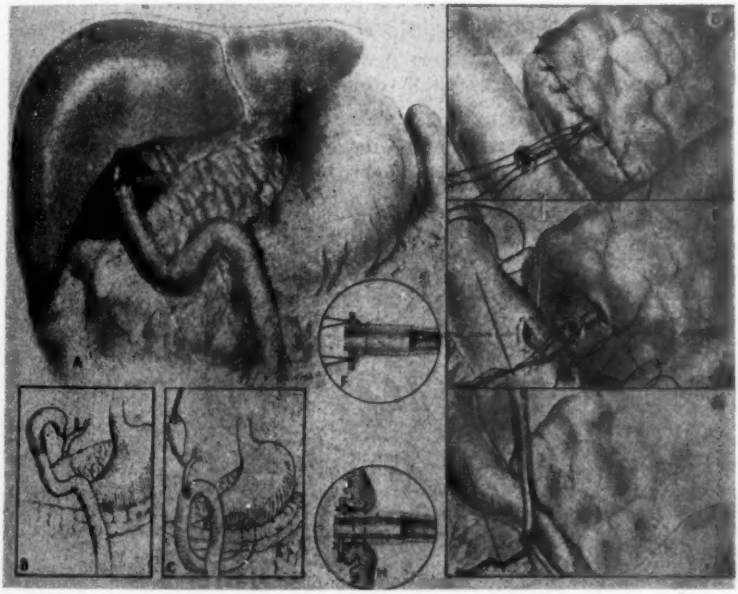
or the duct is relatively small, a non-absorbable suture may be passed through the duct and into the lumen of jejunum to be tied down as a necrosing suture which will result in the development of a fistula between the duct and the bowel. Ligation of the arterial blood supply to the pancreas will decelerate the rate of growth of the tumor and frequently proves quite helpful in prolonging the life of the patient. The accompanying slide (Slide No. 11, please), demonstrates the points of ligation of the anterior-superior pancreatoduodenal artery; the superior pancreatic artery, and the common inferior pancreatoduodenal vessel. It should be realized, however, that these vessels are not always constant. (Lights, please.) Finally, a gastrojejunostomy should be performed because in a certain percentage of patients with malignant lesions in the ampullary area obstruction of the duodenum will develop. If, on exploration, the block dissection appears feasible, then there is a choice of two general procedures. Either a one stage operation, or the procedure carried out in two stages. The one stage procedure is to be favored. However, if the patient has had a long-standing obstructive jaundice and is in poor general condition, they will not tolerate such an extensive procedure at this time and the obstructive jaundice must first be relieved permitting the




SLIDE NO. 12

Shows various procedures proposed for resection of the pancreas.

patient to recover, following which the second stage can be performed with a lower rate of operative mortality. The first stage in no manner simplifies the second stage of the two stage procedure. Because of the poor condition of these patients, the first stage should consist merely of relief of the obstructive jaundice by anastomosing the gallbladder to the jejunum and by taking of the liver biopsy. Liver biopsy should be taken because the microscopic examination might reveal metastases which otherwise could not be demonstrated. A number of operative procedures have been described. (Slide No. 12, please.) In general, the one stage operation and the second stage of the two-stage procedure differ only in certain minor



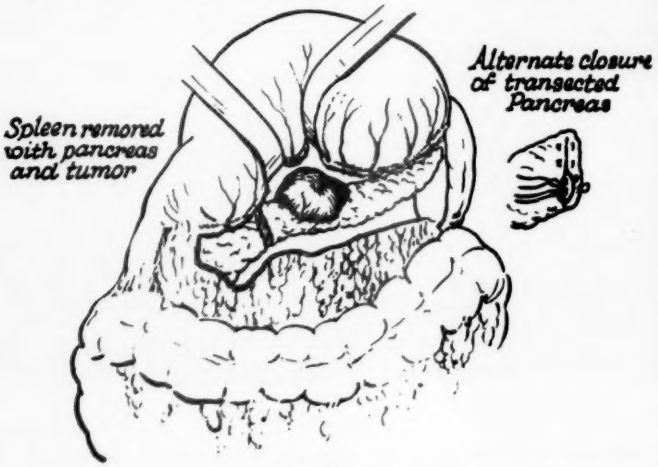
SLIDE No. 13

Illustration of the important principle in resection of the head of the pancreas to protect the liver and pancreas from reflex contamination by stomach contents by use of Roux-Y procedure. The stomach should be anastomosed at least fifteen inches distal to the pancreatic duct anastomosis. This arrangement protects against the formation of stomach ulcers.

details. Without going into the details of the operation, I merely wish to indicate with the following slide (Slide No. 13, please), certain desirable aspects of the completed procedure.

The cholecystjejunostomy, pancreatojejunostomy, and the gastrojejunostomy should be so aligned that the gastric contents have no tendency to regurgitate into either the pancreatic or biliary tract. Implantation of the pancreatic duct into the jejunum is now an accepted part of the operation. It was not included in the original Whipple procedure and the so-called exclusion operation resulted in the development of pancreatic fistulae with high frequency. These extensive operative procedures can be performed with an anticipated mortality of approximately 20 per cent. The postoperative prognosis, however, is not so encouraging and a very small percentage of the patients operated upon are alive at the end of two years; death being due to recurrence of the malignant disease.

Should a malignant tumor occur in the body or tail of the pancreas, it can be resected locally as is illustrated in the accompanying slide. (Slide No. 14, please.) The surgeon should




Slide No. 14

Demonstration of the method of dealing with tumors of the body of the pancreas.

always be prepared to carry out an extensive procedure involving the pancreas, even when a gastric resection is being contemplated, because it might be found that a gastric malignancy might extend into the pancreas requiring total or subtotal resection of this organ. Patients subjected to operations of this magnitude will require careful preoperative and postoperative care with particular care being paid to nutrition, restoration of the blood volume, and the definitive constituents of the blood, as well as preparing the gastrointestinal tract with sulfasuxidine or sulfathalidine in the event damage to the transverse colon results from dissection in the region of the superior mesenteric vessels.

I realize that although this presentation has been rather long, it has also been sketchy, but it will at least serve to re-emphasize the extent to which surgical procedures are now being performed on the pancreas.

THE END

This recording required forty-seven minutes for presentation. This is too long, because it is continuously sustained. It should be slowed up somewhat and broken into two thirty-minute periods. Ordinarily 20–30 minutes discussion will be best received.

# A CLINICAL STUDY OF PHENOSULFAZOLE (DARVISUL) IN ACUTE POLIOMYELITIS*

CLIFFORD G. GRULEE, JR., FREDERIC L. ELDRIDGE, AND G. DAVID FORD

Hope for an effective therapeutic agent for poliomyelitis was given new impetus from the report of Sanders, SubbaRow, Alexander (1948), concerning a new sulfonamide compound (N-(2-thiazolyl)-phenol sulfonamide). These workers found this agent to be effective in treating mice infected with the SK strain of murine poliomyelitis virus.† As a consequence, experiments were extended to include monkeys infected with the Brünhilde III strain of poliomyelitis virus. In these studies, less striking though favorable results were obtained. According to Sanders (personal communication), the compound, which had become known as phenosulfazole, was then given to more than 100 human volunteers, in a dosage of as much as 3.0 gm. daily, for a period greater than 3 months without evidence of toxicity.

The occurrence of an epidemic of poliomyelitis in the vicinity of Galveston, Texas, in the summer of 1948 afforded the opportunity of co-operating with a group of investigators from Columbia University in a study of phenosulfazole in human poliomyelitis.

## MATERIALS AND METHODS

A total of fifty-six patients with poliomyelitis was admitted to the hospital between May 28 and October 23, 1948. The majority of these were from Galveston and surrounding communities. Since phenosulfazole was not available at the beginning of the epidemic, twelve acute cases did not receive it and were not included in the series. In addition, thirteen patients were considered unsuitable for study purposes since they

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†After completion of our study, additional data concerning the drug became available. Several groups of investigators, notably Cox, et al., (1949), Jungeblut (1949), and LoGrippo, et al. (1949), reported their inability to duplicate the original work with mice, Sanders, et al. (1948).

came to the hospital in the convalescent stage. The experimental series then consisted of thirty-one patients who, with two exceptions, were first seen within three days of onset of the second stage, and in whom a presumptive diagnosis could be made on the basis of the clinical symptoms, typical physical findings, and spinal fluid examination. Of these patients twenty-four were white, six were negro, and one was Latin-American. The age range was ten months to thirty-eight years, with fifteen children under eight years, seven children between eight and fifteen years, and nine adults. Nineteen were male and twelve female.

In planning the study, it was decided that phenosulfazole be given to alternate patients irrespective of the type of involvement. Control and treated patients were handled in an identical manner with the exception of the administration of the experimental drug, which was initiated immediately following admission to the hospital. Isolation of all cases was maintained for a period of two weeks after the onset of illness. Care by nurses having previous experience with poliomyelitis was provided through the co-operation of the National Foundation for Infantile Paralysis and the Galveston Chapter of the American Red Cross. Continuous medical care was provided by a team composed of six members of the staff, one of whom was on the ward at all times. Physiotherapy including hot packs, passive, and active exercise as indicated, was begun as soon as the patients were over the acute febrile stage of illness. Members of the orthopedic staff advised in regard to all rehabilitating procedures.

The dosage of phenosulfazole ranged from 200 mg. to 450 mg./Kg./day. The smaller dose was used early in the epidemic, and was increased gradually to the latter figure. An original group of ten patients received on the first day of treatment a calculated dose of 200–300 mg./Kg. by continuous intravenous infusion in 5 per cent glucose solution. In addition, twelve hours after intravenous therapy was instituted, these same patients were given one-half the calculated daily dose orally in three divided doses at four hour intervals. It is apparent then that this low dosage group actually received 150 per cent of the calculated daily dose during the first twenty-four hour period.

For the next three days the entire daily dose was given orally in divided doses at four hour intervals. Because of persistent vomiting in one instance, intravenous therapy was reinstituted. As the study progressed, it was decided to employ the intravenous route only. Eight patients then received a dose of 400–450 mg./Kg./day by continuous intravenous infusion in 5 per cent glucose solution for a period of four days.

With every effort toward objectivity a detailed routine was strictly adhered to in evaluating the progress of the disease in each patient. (1) Careful physical examination was performed several times a day during the acute phase of the illness. Clinical notes and special charts of symptoms and findings showing the progress of the disease were maintained. (2) Neuromuscular examinations were accomplished and recorded daily on specially prepared forms during the acute febrile illness and several times weekly during early convalescence. (3) Record of the blood pressure, as well as pulse and respiratory rates, was kept. (4) Serial lumbar punctures were performed, the first on admission, then on the third, fifth, seventh, and fourteenth hospital days. A constant search for evidences of drug toxicity was made. Daily urinalyses and blood counts twice weekly were carried out. No method for measuring blood concentrations of phenosulfazole was available.

The criteria used in assaying the status of both the treated and control patients were (1) the duration and degree of temperature elevation, including data pertinent to the febrile illness immediately preceding hospitalization; (2) evidence of progression of the disease as measured by paresis; and (3) the cerebro-spinal fluid findings in serial lumbar punctures.

## Results

The duration of temperature elevation, as shown in Table I, ranged from 1 to 13 days. The maximum duration was 7 days for all except one patient who was a paralyzed woman receiving phenosulfazole. Two-thirds of all cases had elevations of temperature persisting 4 to 7 days. The average durations of fever in the control and treated non-paralytic cases were 3.7 days and 3.8 days respectively, while in the control and treated paralytic cases the average durations were 5.6 days and 6.1

days. The average duration of fever of all control cases was 4.5 days, as compared to 5.3 days for all treated cases. It is apparent that phenosulfazole effected no reduction in the duration of the febrile period.

| Duration of Fever (Days) | Total Patients | Treated | | Controls | |
|---|---|---|---|---|---|
| | | Non-paralytic | Paralytic | Non-paralytic | Paralytic |
| 1 | 1 | 1 | 0 | 0 | 0 |
| 2 | 3 | 0 | 0 | 3 | 0 |
| 3 | 3 | 1 | 1 | 1 | 0 |
| 4 | 8 | 2 | 3 | 2 | 1 |
| 5 | 3 | 1 | 0 | 0 | 2 |
| 6 | 6 | 1 | 4 | 1 | 0 |
| 7 | 6 | 0 | 3 | 1 | 2 |
| 13 | 1 | 0 | 1 | 0 | 0 |
| Average of each Category (days) | | 3.8 | 6.1 | 3 7 | 5.6 |
| Average for treated and control groups (days) | | 5.3 | | 4.5 | |
| Average for all groups (days) | | 5 0 | | | |

Table I

*Duration of Fever in 31 Patients with Acute Poliomyelitis, 18 of Whom Received Phenosulfazole.*

The mean temperature elevation in the two groups was not significantly different. Four cases in the control group and four in the treated group had an average temperature of 38° C. or less; nine control and 13 treated cases ranged from 38° to 39° C., and one patient in the treated group had an average temperature elevation greater than 39° C.

On admission there were 10 non-paralytic cases in the control and 12 in the treated group. At the peak of paralytic involvement, 3 of the control patients and six of the treated patients had developed paresis (Table II).

The serial cerebro-spinal fluid findings, including cell count, differential cell count, and protein content were subjected to

statistical analysis and no significant differences in the control and treated groups were found.

| | TREATED | | CONTROLS | |
|---|---|---|---|---|
| | Non-paralytic | Paralytic | Non-paralytic | Paralytic |
| on Admission | 12 | 6 | 10 | 3 |
| At point of Maximum Progression | 6 | 12 | 7 | 6 |

TABLE II

*Incidence of Paralytic Involvement in 18 Patients Treated With Phenosulfazole and 13 Control Subjects.*

The symptoms and signs of possible toxicity to phenosulfazole were vomiting, hematuria, and generalized convulsions. Vomiting occurred in 15 of the 18 treated patients as compared to 4 of the 13 control patients. Hematuria was found in two instances. One patient had microscopic hematuria on the third day of phenosulfazole administration. The other patient had urinary retention and developed gross hematuria during tidal irrigation with 1:20,000 solution of zephiran chloride. The hematuria disappeared when the irrigating fluid was changed to distilled water even though the phenosulfazole therapy was continued. A 16-year old girl, after 30 hours of phenosulfazole by intravenous infusion administered at the rate of 450 mg./Kg./day, developed a generalized convulsion associated with urinary incontinence. The drug was discontinued and no subsequent convulsions occurred. A careful search into the patient's past history revealed no previous convulsive episodes.

## DISCUSSION

The evidence presented that phenosulfazole did not affect the natural sequence of acute poliomyelitis must be evaluated with reference to the characteristic features of developing viral damage to nerve cells. Bodian (1948), has shown in monkeys

that the onset of pathologic changes in the central nervous system occurs early in the preparalytic period. By the first day of paralysis, there is widespread dissemination of the virus in the central nervous system, and parallel pathologic changes are demonstrable. It is probable that by the time the clinical diagnosis is made in human subjects much of the damage to cells of the central nervous system will have already been accomplished. It follows then that little benefit could be expected from even an effective anti-viral agent.

The limitations to a therapeutic experiment in poliomyelitis have been clearly delineated by the Council on Pharmacy and Chemistry of the American Medical Association (Therapeutic Trials Committee, 1949). It is virtually impossible to determine with accuracy the time of onset of the infection. No two cases are entirely similar as to the rate and extent of progression of the pathologic process. In addition, Bodian (1948), has demonstrated that up to 30 per cent of the motor neurons in a given anatomical level of the spinal cord may be rendered functionless before any clinical signs of neurologic involvement become apparent. This means that progression of the disease process may occur without being demonstrable by the most searching physical examination.

The findings in our study seem more significant when considered along with the results obtained by Lawson (Grulee and Lawson, 1948). In the summer of 1948 this worker undertook a clinical study of phenosulfazole at Winston-Salem, North Carolina. Considering only those patients who had no paralysis at the time of admission to the hospital, paralysis developed in 5 of 18 treated patients, and 5 of 17 control subjects. By combining his results with those of our study, a total experience with 57 non-paralytic patients is obtained. Of the 30 who received the drug, 11 developed paralysis; of the 27 control patients who did not receive the drug, 12 became paralyzed. Furthermore, there was no significant difference in the duration of fever between treated and untreated patients in either series. It can be concluded that clinical experience supports the findings in several recently published laboratory studies by Cox, et al. (1949), Francis and Brown (1949), Jungeblut (1949), LoGrippo, et al. (1949), and Weiland Warren

(1949), which indicate that phenosulfazole is not active therapeutically against the viruses of poliomyelitis.

SUMMARY

A series of 31 cases of acute poliomyelitis was studied, of which 18 patients received phenosulfazole (Darvisul) and the remainder served as controls. Doses of this drug up to 450 mg./Kg./day administered intravenously and continued for 4 days were therapeutically ineffective. Toxic reactions included vomiting in approximately 80 per cent of patients and one instance each of hematuria and a generalized convulsion. Theoretical limitations to the effective use of anti-viral substances and practical difficulties in their clinical evaluation are briefly discussed. It is apparent from both clinical and laboratory studies that an effective anti-viral agent for poliomyelitis is yet to be found.

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# ACCIDENTAL MICROSCOPIC FINDING OF TOXOPLASMA IN HUMAN BLOOD

S. Edward Sulkin, C. H. Lodowski and Lester W. Hartman*

Although the pathogenicity of toxoplasma for lower animals has been recognized for many years, toxoplasmosis as a disease in man has been an established fact only within the last 10 years (Wolf et al., 1939, 1940). Sufficient evidence has been presented to show that these protozoan parasites are responsible for certain forms of congenital encephalitis (Wolf et al., 1939, 1940), acute nonsuppurative encephalitis (Sabin, 1941) and a spotted fever-like disease (Pinkerton and Henderson, 1941). Although dissemination of the parasite within the body presumably occurs by way of the bloodstream, toxoplasma are only infrequently found in smears of blood of experimentally infected animals, and have rarely been demonstrated in human blood even by animal inoculation. Pinkerton and Henderson (1941) reported the first instance of experimental transmission of toxoplasmic infection to guinea pigs by inoculation with human blood obtained during life. In a few instances, toxoplasma-like structures have been observed in the blood of man. Castellani (1914) described structures which he believed to be toxoplasma in smears of the blood of a child who died of a disease characterized by severe anemia, prolonged fever, splenomegaly and leukopenia. Wenyon (1923), however, suggested that the structures observed by Castellani (1914) were degenerating leukocytes or yeast cells. Fedorovitch (1916) observed similar structures in blood films from a child who presented similar clinical symptoms. Wenyon (1923) again refused to accept these structures as protozoa.

More recently, Drenowski (1947) reported the accidental microscopic finding of toxoplasma in human blood. This observation was made during the course of routine examinations of blood films for malarial parasites. No data were available

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concerning the clinical course of the individual from whom the blood specimen was obtained nor has it been definitely established that the organisms were toxoplasma. Since the reports of Castellani (1914) and Fedorovitch (1916) appeared long before the human disease was differentiated, it cannot be stated with assurance that the structures seen in the blood were toxoplasma. The possibility that they were toxoplasma, however, cannot be excluded.

Toxoplasma have been seen only rarely in blood films even from severely infected animals (Sabin, 1942a, Sulkin, 1948). Furthermore, there is no evidence that multiplication of these protozoan parasites occurs in erythrocytes or leukocytes, yet there is evidence that walls of blood vessels are frequently invaded (Sabin and Olitsky, 1937). By mouse inoculation, these investigators demonstrated organisms in the blood of Rhesus monkeys following intracutaneous injection of toxoplasma. Sabin and Warren (1942) demonstrated toxoplasma in the blood of dogs with mild infections following subcutaneous inoculation.

The present report is concerned with the accidental observation in blood films of toxoplasma-like structures which proved to be toxoplasma on the basis of serologic studies of the patient's serum.

## Case Report

The patient, an 11-month-old white male, was admitted to the pediatric service on December 6, 1948, because of 4 right-sided facial convulsions during the week prior to admission. Physical findings included a well-developed white male child who did not appear acutely ill. Other than head contour suggestive of hydrocephalus there were no abnormal physical findings. A neurologic examination by Dr. Thomas W. Farmer revealed no abnormalities and fundoscopic studies by Dr. E. G. Grafton revealed no evidences of chorioretinitis. Roentgenograms of the skull showed no evidence of intracranial calcification. Laboratory studies revealed a moderately severe hypochromic microcytic anemia, 10 lymphocytes and 2 polymorphonuclear leukocytes in the spinal fluid, elevated protein content in the spinal fluid and spinal fluid pressure above 300

mm. of water. Past history revealed that the patient had been a feeding problem for the first 6 months of life.

During the first 3 days in the hospital the fever subsided. Two right-sided convulsions involving the face and right arm occurred during the first week of the hospital course. The remainder of the hospital course of 8 weeks duration was characterized by an excellent response of the anemia to iron therapy, absence of fever and convulsions, weight gain and no evidences of mental or personality changes. Several lumbar punctures, however, revealed a pressure above 300 mm. of water. There was evidence of slight increase in head circumference during the 8-week hospital course. Two electrocardiograms showed tracings compatible with the Wolffe-Parkinson-White syndrome and an electroencephalogram by Dr. Paul M. Levin showed adnormal activity in the left central area. Pneumoencephalograms performed at the suggestion of Dr. Levin revealed slight internal hydrocephalus and widening of the suture lines of the skull which were not apparent on the routine skull films prepared previously. Six months after discharge from the hospital the patient was completely asymptomatic and no significant increase in the head circumference had occurred.

Toxoplasma-like structures were observed during the course of a routine examination of a blood film prepared from this patient on December 16, 1948. The observation was made by Mr. Henry Cobble, laboratory technician, and the slide was referred to this laboratory for study. Structures having all the characteristics of toxoplasma were seen. The organisms were crescentic and pyriform with pale blue staining cytoplasm and large peripheral nuclei. In addition, the structures closely resembled known strains of toxoplasma of human origin which are being maintained in this laboratory. Similar structures were seen in subsequent blood films prepared from this patient, and in such instances, these bodies were found only after long search of the preparations.

It is recognized that diagnosis of toxoplasmosis can be established only by certain laboratory procedures involving demonstration of organisms resembling toxoplasma in body fluids or tissues, isolation of toxoplasma by animal inoculation, and by demonstration of specific antibodies. Accordingly, attempts

were made to demonstrate these protozoan parasites in blood and spinal fluid by animal inoculation. An inbred stock of Swiss mice was used in these experiments. To our knowledge, no spontaneous infections with toxoplasma had ever occurred in this mouse colony. Although guinea pigs would probably have been the animal of choice, rabbits were also used in an effort to demonstrate toxoplasma in blood and spinal fluid from this patient, though it should be pointed out that occasionally an immune rabbit is encountered while doing routine neutralization tests. The methods employed were similar to those described by Sabin (1942a). All attempts to demonstrate toxoplasma in these specimens, even by resorting to the "blind passage" technique, were negative. Since rabbits which recover from infections induced even by intracutaneous injection of toxoplasma often develop immunity (Sabin, 1942a) all rabbits used in these experiments were given challenge intradermal

NEUTRALIZATION TEST WITH L.M. STRAIN OF TOXOPLASMA

Developing lesions traced on the 7th day.

| Mixtures injected Intracutaneously On the back of the same rabbit | Dilution of Toxoplasma-infected tissue in mixtures | | | |
|---|---|---|---|---|
| | 1:20 | 1:100 | 1:1000 | 1:10,000 |
| Toxoplasma + Serum of Sibling I of Patient L.D. | | | | |
| Toxoplasma + Tyrode's solution (Control) | | | | |
| Toxoplasma + Serum of Sibling II of Patient L.D. | | | | |
| Toxoplasma + Serum of Father of Patient L.D. | | | | |

○ = Extent of lesion

● = Area of necrosis

Scale: Centimeters

0 1 2 3 4 5 6 7 8 9 10

injections of a 1:10, 1:100, and 1:1,000 dilution of brain tissue from mice infected with the L. M. strain* of toxoplasma. Indurated skin lesions with hemorrhagic necrotic centers developed in each instance.

Serologic tests for toxoplasmosis were conducted on a number of blood specimens from the patient and on specimens obtained from the parents and 2 siblings. The complement fixation test used was performed with an improved antigen prepared from infected chick embryo tissue similar to that recently described by Warren and Russ (1948) and also developed independently in this laboratory. The L. M. strain of toxoplasma of human origin (Paige et al., 1942) had been maintained by

NEUTRALIZATION TEST WITH L.M. STRAIN OF TOXOPLASMA

Developing lesions traced on the 7th day.

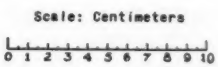
| Mixtures injected Intracutaneously On the back of the same rabbit | Dilution of Toxoplasma-infected tissue in mixtures | | | |
|---|---|---|---|---|
| | 1:20 | 1:100 | 1:1000 | 1:10,000 |
| Toxoplasma + Serum of mother of Patient L.D. |  | |  |  |
| Toxoplasma + Tyrode's solution (control) | | | |  |
| Toxoplasma + L.D. serum (15th day of disease) | | |  |  |
| Toxoplasma + L.D. serum (30th day of disease) | | |  | — |
| Toxoplasma + L.D. serum (6th week of disease) |  |  | — | — |

◯ = Extent of lesion

● = Area of necrosis

— = No lesion

Scale: Centimeters




*This strain was obtained through the courtesy of Dr. Abner Wolf.

intracerebral passage in white Swiss mice and was in its four-hundred and tenth passage when successfully cultivated in embryonated eggs in September, 1947. This strain has since been maintained in mice by intracerebral inoculation and in developing chick embryos by chorioallantoic inoculation. The criteria used in interpretation of the toxoplasma neutralization test of Sabin and Ruchman (1942) were those suggested by Sabin (1942b). The results of the neutralization tests are summarized in the accompanying charts.

A serum specimen obtained from the patient on the fifteenth day of the disease, at the time the toxoplasma-like structures were observed, did not contain neutralizing antibodies for toxoplasma and failed to fix complement with the toxoplasmic antigen prepared from the infected chorioallantoic membrane of embryonated eggs. The results of a second neutralization test conducted on a serum specimen obtained on the thirtieth day of the disease were equivocal and the complement fixation test was positive at a serum dilution of 1:16. A third specimen obtained 5 weeks after the patient was admitted to the hospital contained neutralizing antibodies and fixed complement at a dilution of 1:64 with the toxoplasmic chick embryo antigen. The recently described cytoplasm-modifying dye test of Sabin and Feldman (1948) was also positive with this serum sample. A serum specimen obtained from the mother of this patient on December 17, 1948, was positive in the neutralization test and was also positive in the complement fixation test; a 1:64 dilution of the serum fixed complement in the presence of the toxoplasmic antigen. Similarly, the serum from the 3-year-old sister (sibling II) of the patient showed neutralizing antibodies in the toxoplasma neutralization test. The complement fixation test was negative. Serum specimens from the father and from a 2-year-old sister (sibling I) were negative by both tests.

## Summary

The present report is concerned with the accidental observation of toxoplasma in blood films from a proved case of toxoplasmosis. The identity of the protozoan parasites observed in the blood films was established on the basis of serologic studies of the patient's serum. The serum of the child's mother and

one of two siblings contained toxoplasma-neutralizing antibodies.

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# A NEW TEST TUBE IDENTIFICATION TAG

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Laboratory workers often are hampered by the lack of an accurate and convenient method of labeling small test tubes such as those used in serological tests.

To overcome this difficulty, the writer has developed a detachable metal tag which, when attached to a test tube, definitely identifies that tubes from other tubes.

The tag is prepared from a thin, flat, rectangular piece of malleable metal which varies in size according to the outside diameter of the tube with which it is to be used.

For a precipitin tube 7 mm. in outside diameter, the flat piece of metal measures 18 mm. long and 11 mm. wide. Two parallel slits, 10 mm. long and 6 mm. apart, are made lengthwise on the flat piece of metal. The distance between the two slits is wide enough to allow an identifying number to be stamped in the space (Fig. 1-a). A tapered shaft is inserted in the two slits to arch the center section and the two long sides of the rectangular piece, so that a ring is made (Fig. 1-b). The center section forms one-half of the ring and the long sides of the rectangle, immediately above and below the center section, form the other half (Fig. 1-c and d). These tags are numbered consecutively. They can be used over and over again, and are not affected by immersion of the tagged test tubes in water baths.

Tags similar to those for precipitin tubes (Fig. 1-e), can be made for tubes of different sizes. The tag measurements will vary in proportion to the outside diameter of the tube. For narrow agglutination tubes 10 mm. in outside diameter, the flat piece measures 24 mm. by 12 mm., with two 17 mm. lengthwise parallel slits 8 mm. apart. For regular agglutination tubes 13 mm. in outside diameter, the flat piece measures 25 mm. by 13 mm.. with two 18 mm. lengthwise parallel slits 9 mm. apart. For Wasserman tubes 15 mm. in outside diameter,

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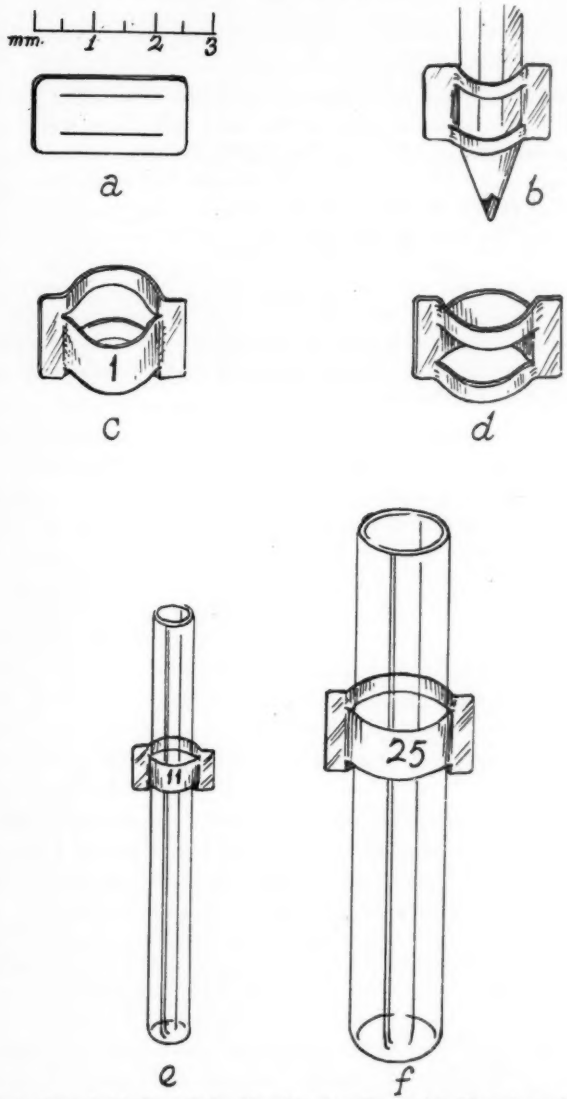


FIG. 1: *Detachable Test Tube Identification Tags.* (a) Flat piece of metal with two parallel slits; (b) A tapered shaft inserted in tag; (c) Front view of numbered tag; (d) Rear view of numbered tag; (e) Tagged precipitin test tube; (f) Tagged Wasserman test tube.

the flat piece measures 27 mm. by 13 mm., with two 20 mm. lengthwise parallel slits 9 mm. apart.

When a test tube intended for labeling is inserted in the tag, the short, rectangular sides of the tag support the stress upon its curved section. By pressing upon the tag, the degree of curvature may be varied to compensate for slight deviations occurring in the outside diameter of tubes, thus insuring a snug fit for the tag on each test tube.

## Summary

A detachable metal tag is described. This tag, when attached to a test tube, serves as a label which definitely identifies that test tube from other tubes.

# A NON-FERMENTABLE SOLID MEDIUM FOR THE CULTIVATION OF ENDAMOEBA HISTOLYTICA

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A medium highly favored during the past several years for the cultivation of *Endamoeba histolytica* has been the Bacto endamoeba medium of Difco. Cleveland and Sanders (1930) and Cleveland and Collier (1930) found a liver infusion agar of this formula to be almost specific for *E. histolytica*. One of the best media for practical diagnostic purposes according to Spector (1935–36) is the same medium overlaid with a 1:6 serum-saline mixture.

For isolation and maintenance of *E. histolytica* cultures liver infusion agar has one drawback. The liver infusion contains a substance fermentable by the bacteria that grow in association with the amoebae. This leads to gas bubbling that splits the medium and permits the amoeba to slide down and out of reach. Recovery of the amoeba becomes difficult and cumbersome, and this impedes transplant, isolation from stools, or estimation of numbers present.

The purpose of the present study is to determine whether prefermentation of the medium would eliminate this difficulty. Prefermented media were tested for their support of growth of the endamoeba and for a diminution in gas production by the associated bacteria.

Two experimental types of endamoeba media were kindly provided by the Difco Laboratories. In one of these, fermentable substances had been removed by a yeast and in the other by *Escherichia coli*. This material was supplied in the form of a dehydrated liver infusion agar, prefermented as stated. It had been made at the request of Dr. A. Packchanian in 1936 and supplied to him for such study as is here reported.†

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*From the Department of Bacteriology, The University of Texas Medical Branch, Galveston, Texas. Received for publication November 16, 1949.

†Acknowledgment is made to Dr. A. Packchanian who supervised this study and provided the cultures and media.

## Experimental Methods and Results

The *E. histolytica* was cultured on three media: one nonfermented, one prefermented with yeast, and one prefermented with *E. coli*. The first mentioned, used as a control, was the dehydrated Bacto liver infusion agar of the market (Endamoeba medium No. B 35). This will be referred to in this paper as medium A. The experimental media were Difco's dehydrated Bacto liver infusion agar No. 286396 in which the beef liver infusion had been fermented by yeast (medium B), and Difco's dehydrated Bacto liver infusion agar No. 286397 in which the beef liver infusion had been fermented by *E. coli* (medium C).

These media were made up into slants (6 cc. per test tube), each tube of which was overlaid with 2.5 cc. of a 1:10 dilution of rabbit serum in normal buffered saline (Craig, G. M., 1936) and treated with as much sterile rice powder as could be picked up on a moistened 5 mm. loop.

We happened to have in the laboratory a strain of *E. histolytica* growing with bacteria that produced voluminous gas on the regular endamoeba media. This endamoeba-bacteria mixture was selected as test organism.

The three media, nonfermented, yeast fermented, and *E. coli* fermented, were handled in identical manner. Identical inoculum was applied to each, 0.8 cc. of the *E. histolytica* mixed culture from laboratory stock.

Detail of the experiment was as follows. Three tubes each of the three media were set up; 1A, 2A, and 3A with the nonfermented medium, 1B, 2B, and 3B with the yeast fermented medium, and 1C, 2C, and 3C with the *E. coli* fermented medium. All were inoculated with the *E. histolytica*-bacteria complex and incubated for 6 days. After 24 hours and on each succeeding day during this period a quantitative estimation was made of the gas formed.

After 24 hours incubation the original tubes 1A, 1B, and 1C were each transferred to three new tubes of media, 4A, 5A, 6A; 4B, 5B, 6B; and 4C, 5C, 6C, and incubated. All of these tubes likewise were examined for gas formation every day for six days and the amount of gas recorded each day. Three new transfers were made from each of the original tubes 2A, 2B,

and 2C after 48 hours incubation, and from the original tubes 3A, 3B, and 3C after 72 hours incubation, and similarly examined.

The amount of gas formed in the tubes was measured in two ways as a check of one upon the other. One method was to estimate the amount of gas in each tube by a series of plus signs (+, ++, +++, etc.). The other method was to measure with a millimeter ruler the amount of gas formed at the bottom of the tube or in broad splits and to add in a millimeter estimate of the amount of gas contained in bubbles. The readings from these two techniques checked to such degree that only one of the records need be presented here.

The amount of gas formed in the variously fermented liver infusion agars by the organisms growing in association with this strain of *E. histolytica* showed up as follows on the individual tests. The original inoculated tubes of the nonfermented medium (in triplicate) showed gas measuring 12 mm., 12 mm., and 13 mm. after 24 hours; 18 mm., 20 mm., and 19 mm. after two days; 22 mm., 23 mm., and 20 mm. after three days; 18 mm., 19 mm., and 16 mm. after four days; 13 mm., 15 mm., and 12 mm. after five days; 11 mm., 13 mm., and 8 mm. after six days.

This nonfermented medium's gas production is seen to have increased for a period of 2 to 3 days when it reached the maximum of more than 22 mm. After the third day there was a very gradual decrease, 19.5 mm. of gas still remaining on the sixth day. (See Fig. 1.)

The agar was so disrupted with bubbles and breaks from this gas formation that the rice powder and amoebae slipped to the bottom of the tube as shown in the A tubes of Figure 2. To recover the organisms a pipette had to be passed through a layer of agar, often becoming plugged before the amoebae were reached.

The tubes of yeast-fermented medium formed contrastingly little gas. The latter (also in triplicate) showed gas measuring 4 mm., 0, and 0 after 24 hours; 5 mm., 4 mm., and 0 after 2 days; 7 mm., 3 mm., and 0 after three days; 4 mm., 1 mm., and 0 after 4 days; 2 mm., 0, and 0 after 5 days; 2 mm., 0, and 0 after 6 days.

Here again gas production reached a maximum after the third day of incubation but in only 4 mm., quantity, approximating about 1/8 that of the nonfermented medium. (See Fig. 1.)

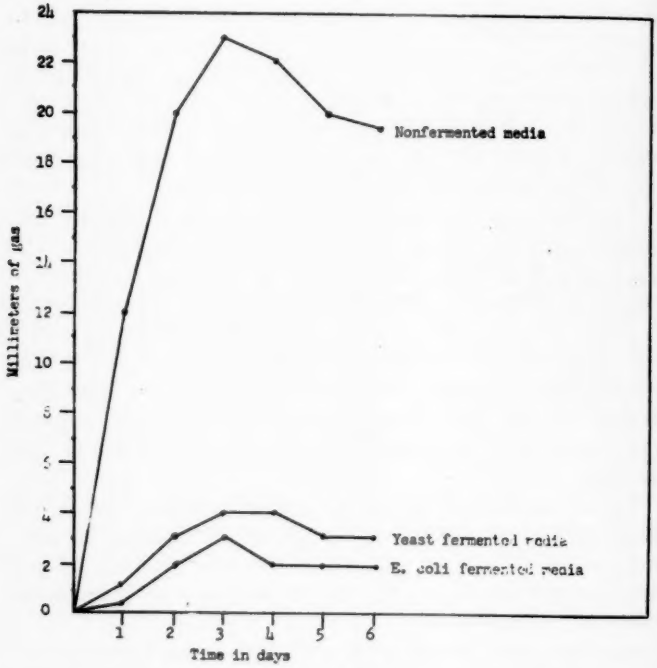


FIG. 1. Amount of gas formed by Endamoeba symbionts in nonfermented liver infusion agar. (Ordinates represent average of 12 tests.)

The gas in this instance formed only one or two small bubbles as shown in $B_2$ of Figure 2, or perhaps a layer of from 1 to 4 mm. at the bottom of the tube. This left the rice-amoeba layer at the bottom of the agar slant undisturbed and recovery of the organisms was a simple procedure.

The tubes of *E. coli*-fermented medium showed gas measuring 0, 0, and 0 after 24 hours; 0, 4 mm., and 4 mm. after 48 hours; 0, 3 mm., and 5 mm. after 3 days; 0, 0.5 mm., and 3 mm.

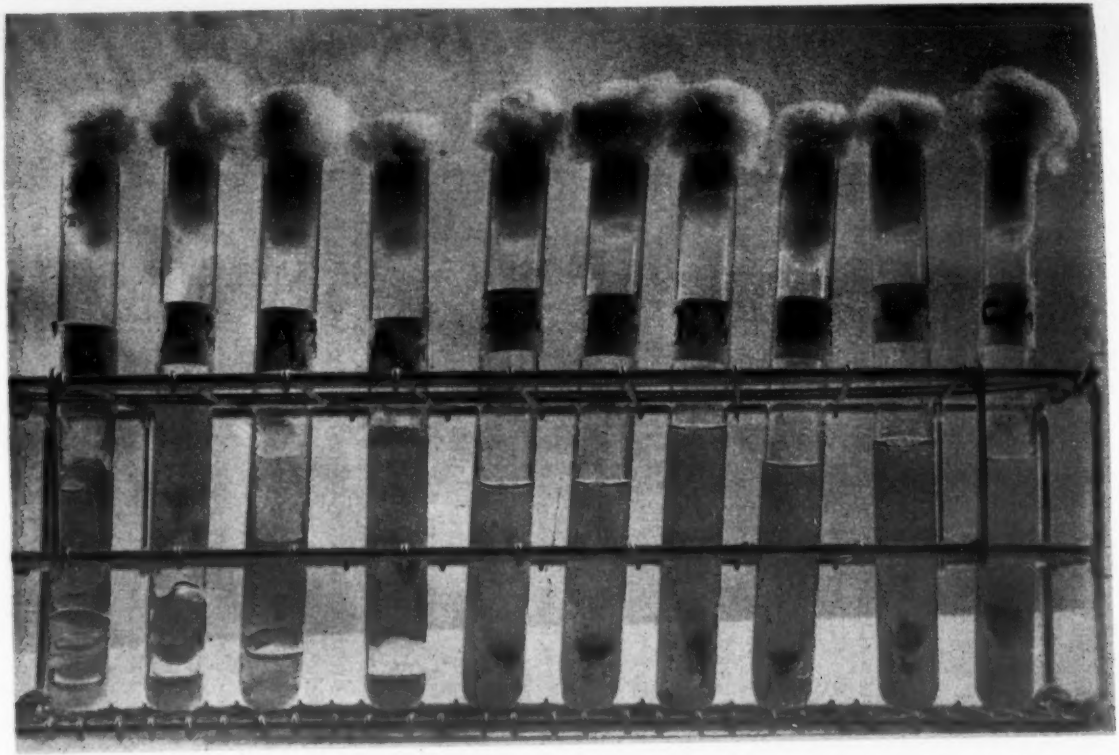

FIG. 2. Tubes showing gas formation in nonfermented medium A, and absence of gas in prefermented media B and C. Note that in first three tubes of media A most of the rice-amoeba mixture has slipped down the slant and is located either in the bottom of tube or caught between layers of agar separated by gas. The small bubble in $B_2$ and $C_2$ has not disturbed the rice-amoeba mixture.

after 4 days; 0, 0.5 mm., and 1 mm. after 5 days; and 0, 0.5 mm., and 0.5 mm. after 6 days.

That is to say, there was a reduction in the quantity of gas formed about equal to that with the yeast-fermented medium. The rise in gas production reached a maximum the 2nd or 3rd day and decreased very slightly from the 3rd to the 6th day. It reached a maximum average of about 3 mm. (See Fig. 1.)

The subcultures made from the originals after 24, 48, and 72 hours, and incubated for 6 days gave similar results. In the 27 subcultures of the non-fermented medium A, the yeast fermented medium B, and the *E. coli* fermented medium C, the average amount of gas formed in the 9 tubes of each respective medium over a period of six days was respectively 20.8 mm., 3.6 mm., and 1.8 mm. The decrease in gas formation in the prefermented medium B as compared with the nonfermented medium A, was 82.7%, and in the prefermented medium C, 91.4%, figures approximating those of the preceding series.

These similar results show that repeated subculture on the prefermented media did not affect the flora. The endamoebae and their bacterial associates grow and react in just the same manner after repeated subculturing. Prefermentation continued to eliminate the greater part of the gas ordinarily formed by the Endamoeba symbionts.

The most significant readings in these several series of tests are those taken after 48 to 72 hours of incubation, the usual transfer period for amoebae. Gas occurred in all of the tubes of nonfermented medium A with an average quantity of 18.3 mm. per tube. In the yeast fermented medium B, three-fourths of the tubes showed gas but with an average quantity of only 2.7 mm. per tube. In the *E. coli* fermented medium C, half of the tubes showed gas with an average quantity of 1.7 mm. per tube. (See Table I.)

In the nonfermented medium A, the gas production was of such quantity as to break up the slant at the bottom of which the amoebae were growing. In some instances this allowed the amoebae to slip down and out of reach, as had previously been

observed, and in others forced the amoebae up beyond the serum saline overlay and interfered with their viability.

TABLE I

*Gas formation by Endamoeba-bacterial complex and by E. coli in prefermented and nonfermented media.*

| | Gas formation in 48 hours by | | | | | |
|---|---|---|---|---|---|---|
| | Endamoeba symbionts | | | Escherichia coli | | |
| | Total No. of Tubes | Tubes with Gas | Ave. mm. of gas per tube | Total No. of Tubes | Tubes with Gas | Ave. mm. of gas per tube |
| Nonferm'd Media A | 12 | 12 | 18.3 | 12 | 12 | 8 |
| Yeast " " B | 12 | 9 | 2.7 | 12 | 0 | 0 |
| E. coli " " C | 12 | 6 | 1.7 | 12 | 1 | 0.1 |

In the prefermented media B and C on the other hand, so little gas was formed that the slant was never disturbed. Gas formation produced but a few bubbles in the base of the agar slant as in $B_2$ and $C_2$ of Figure 2. If it collected at the bottom of the tubes at all, this was in very small amounts and raised the agar slant only as an intact whole. This gas did not break up the slant of the agar and disturb the amoeba-rice mixture at the bottom.

An additional experiment was set up to find whether the residium of gas production in the prefermented media was due to incomplete action of the fermenting agents employed or to a differently fermenting agent having entered with the amoeba-bacterial complex. The same media were used: non-fermented, yeast fermented, and *E. coli* fermented, but this time with *Escherichia coli* as the test organism. The procedure was identical with that in the amoebae experiment.

The findings in this experiment were as follows. Results of 12 tests with nonfermented medium A showed gas in all tubes with an average of 7 mm. of gas evident after 24 hours, 9 mm. after 48 hours, 8 mm. after 3 days, 6 mm. after 4 days, 5 mm. after 5 days, and 4 mm. after 6 days. Results of 12 tests with

the yeast fermented medium B showed a small bubble in 6 tubes (like that of $B_2$ of Fig. 2). These bubbles usually disappeared after the second or third day of incubation. Results of 12 tests with the *E. coli* fermented medium C showed 1 mm. of gas in 1 tube after 48 hours of incubation. This gas disappeared at the fourth day of incubation. Results of this experiment are represented in Figure 3.

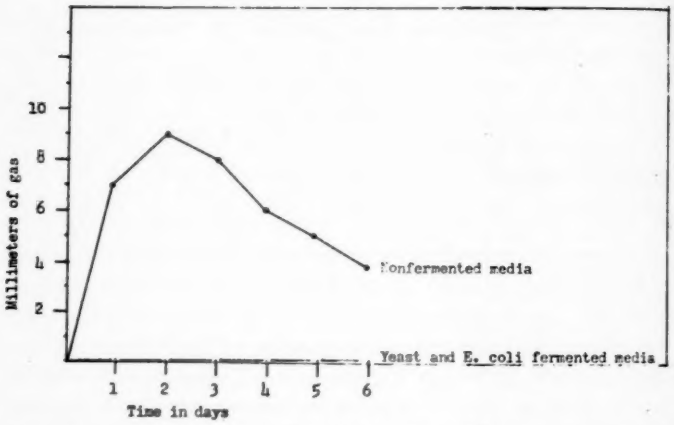


FIG. 3. Amount of gas formed by Escherichia coli in nonfermented and prefermented liver infusion agar. (Ordinates represent averages of 12 tests.)

At 48 hours, the ordinary period of amoeba transfer, the amount of gas produced in the various media averaged as follows. All 12 tubes of nonfermented medium A inoculated with *E. coli* showed gas with an average amount of 8 mm. The 12 tubes of yeast fermented medium B inoculated with *E. coli* showed no gas in any instance, or only immeasurable amounts in the form of small bubbles. None of the tubes of *E. coli* fermented medium C inoculated with *E. coli* showed gas except one and it only 1 mm., an average of 0.083 mm. per tube. (See Table I.)

In this *E. coli* experiment, practically all gas formation was prevented in the prefermented media. Less than half as much

gas was produced in the nonfermented medium as had been produced by the *Endamoeba*-bacterial complex.

The gas that was still to be formed by the *Endamoeba* symbionts in the liver infusion agar after prefermentation by yeast or *E. coli* must therefore be attributed to a factor in the liver infusion or other components of the medium which is not fermentable by yeast or *E. coli*. The bacterial symbionts of amoeba have fermentative capacities that the *E. coli* does not.

This substance must be present in relatively small amount for the gas produced by the *Endamoeba* symbionts in the prefermented media was only about 1/7 or 1/8 as much as that produced in the nonfermented medium. It might be removed through the use of other prefermenting agents, but the gas it produces is too small in amount to warrant that attempt. It does not cause the technical difficulty under study.

The prefermentation of the media did not notably affect the growth of the amoeba. The number of organisms that could be recovered from either of the prefermented media approximated that from the endamoeba media regularly marketed.

Counts were made from a drop of culture taken from the vicinity of the rice powder. The average numbers in nonfermented media A, yeast fermented B, and *E. coli* fermented C were respectively 15, 8, and 12 per high power field after 24 hours; 20, 12, and 15 after 48 hours; and 9, 10, and 8 after 72 hours.

## Discussion

The results of these experiments indicate that complete elimination of the gas formation in the yeast prefermented liver infusion agar occurs in a fourth of the cases, and in the *E. coli* prefermented liver infusion agar in half the cases. In the yeast prefermented medium there is an 85% reduction in the remaining three-fourths of the tubes, and in the *E. coli* prefermented medium a 90.3% reduction in the remaining half of the tubes.

The small amount of gas that occurs in the prefermented media does not disturb the slant surface in any way, and leaves the growth of the amoebae intact for recovery at the bottom of an unbroken slant. Amoebae grown in media in

which the gas factor can be controlled are therefore more readily transferred. There is also greater assurance that the inoculum contains a quantity of organisms.

Instead of a series of drops having to be transferred, one drop suffices. Difficulties encountered with the nonfermented medium are entirely overcome. Gas no longer breaks up the slant and causes the organisms to slip into cracks or become trapped at the bottom of the tube by resettling of the agar layers.

Elimination of the gas factor is of considerable value in the routine diagnosis of amebiasis through feces culture. Lucas (1945), not having this facility at hand, finds that "In some cases several amoebae may be found in most fields while in others considerable search must be made before a single individual is discovered."

Examination of a series of drops or even repeated subcultures is recommended by Craig (1934) before a culture can be called negative. The additional subculture involves an extra 24 hours before diagnosis. If the slants are not disrupted by gas, this additional time consumption becomes unnecessary and false negatives are less likely to occur.

## Conclusions

1. The gas formed in liver infusion agar by the *Endamoeba histolytica*-bacteria complex can be eliminated or greatly reduced if the liver infusion to be incorporated in the medium is first fermented by yeast or by *Escherichia coli*.

2. Such prefermentation of the liver infusion does not affect growth thereon of the *Endamoeba histolytica*.

3. A substance not removed by this prefermentation remains and is fermentable by the endamoeba symbionts, but is present in too small a quantity to present practical difficulties.

4. Prefermentation of the medium increases the number of organisms recoverable for transfer and permits more rapid diagnosis of amoebiasis.

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# A NOTE ON THE FAILURE OF HYPNOSIS IN LABILE ESSENTIAL HYPERTENSION

ALFRED LANE AND ARTHUR RUSKIN*

For many years it has been known that ordinary sleep is associated with a reduction in normal and hypertensive blood pressures. Thus, Brooks and Carroll in 1912 noted average drops in systolic pressures of 16 to 24 mm. of mercury in the first 2 hours of sleep as compared with the maximal readings at 4 to 5 o'clock in the afternoon. The higher the systolic pressure the greater was the fall, reaching 45 mm. in severe hypertensive cases. Hypnotics did not lower the blood pressure further. It is also well-known that the sodium amytal test shows drops of blood in labile hypertensive patients similar to those of sleep and, frequently, those of sympathicolytic drugs.

Several authors, mostly German, have claimed beneficial effects of hypnotherapy in isolated instances. No known series of hypertensive patients has been found in the literature in which the effects of relaxation under hypnosis were systematically studied. Since one theory of hypnosis, that of the Pavlovian school of neuropsychiatrists, relates it to normal sleep through midbrain and hypothalamic mechanisms, it was considered especially desirable to compare the effects of relaxation under hypnosis with those of normal sleep, sodium amytal and etamon (tetraethyl ammonium).

## METHODS

Seven subjects with uncomplicated essential hypertension were successfully and repeatedly (5 to 10 times) hypnotized in the sitting and lying positions to the stage of deep hypnosis. After several sessions the standard conditioning phrases resulted in rapid deep hypnosis, as checked by the usual tests. The patients were told, both in and out of the hypnotic state, that the utmost relaxation and peace of mind was necessary

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for the purpose of achieving a possible hypotensive result. Positive suggestions, and posthypnotic suggestions of extreme leaden limpness were repeatedly droned into the patients' ears during the deeper half hour trances.

Numerous control blood pressures were available both at the start of the hypnotic sessions and at various periods when the same patients were studied with other therapeutic regimens in the Hypertensive Clinic. Blood pressures were also repeatedly taken during and after hypnosis. Sodium amytal tests were performed in the usual way, 0.2 gm. every hour for 3 hours being preceded by numerous control and followed by frequent follow-up readings until the "floor" readings were passed. Etamon tests were performed with 0.5 gm. of tetraethyl ammonium chloride intravenously, and control readings following again beyond the "floor" values. In 3 of the same patients we were able to take blood pressure readings 1 to 2 hours after the onset of natural sleep, in 3 others in the deep sleep following the more profound hypnotic trances.

## Results

Control blood pressures and blood pressures during hypnosis, hypnotic sleep and natural sleep are recorded as medians or range averages of those periods in Table I. Floors of etamon and sodium amytal tests are appended in the same table for

Table I

*Summarizing Data on the Effect of Hypnosis, Sleep, Sodium Amytal, and Etamon on Essential Hypertension.*

| Patient | Age | Control | Hypnosis | Sleep | Post-hypnotic sleep | Etamon Floor | Sodium Amytal Floor |
|---|---|---|---|---|---|---|---|
| J.L.S. | 47 | 200/115 | 200/115 | 190/110 | ..... | 140/100 | 160/110 |
| R.B. | 58 | 150/120 | 145/115 | 135/100 | ..... | 120/ 95 | 145/100 |
| M.G. | 44 | 170/105 | 170/105 | ..... | ..... | 130/ 85 | 130/ 95 |
| L.J. | 46 | 175/110 | 175/115 | ..... | 160/105 | 135/100 | 140/105 |
| A.C. | 49 | 180/125 | 185/130 | ..... | 155/115 | 130/ 85 | 160/ 90 |
| P.J. | 50 | 165/110 | 165/110 | ..... | 140/ 90 | 125/ 95 | 140/100 |
| N.O. | 44 | 210/130 | 205/125 | 180/105 | ..... | 150/ 90 | 190/115 |

comparison. It will be seen that while some correlation exists between drops in systolic, diastolic and presumably, mean, blood pressures as a result of sleep, sodium amytal and etamon, that deep hypnosis with all possible efforts at physical and mental relaxation had no such hypotensive effect. The blood pressures remained remarkably constant during and after hypnosis, as compared with the control readings.

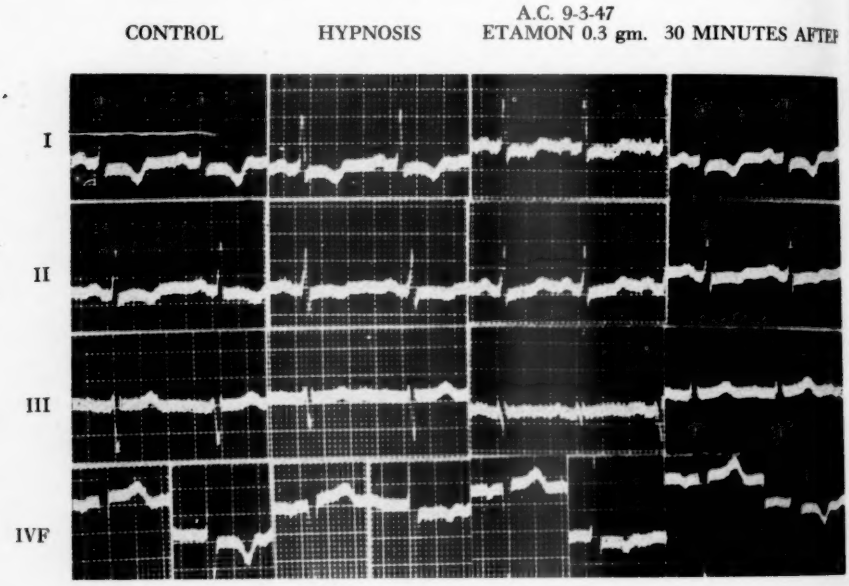


FIG. 1. Electrocardiograms of case A.C. (see Table I) showing control left ventricular strain pattern when the blood pressure average was 220/130. Blood pressure under hypnosis was 215/130 and the only change in the electrocardiogram was a less negative $T_4$. 0.3 gm. of tetraethyl ammonium lowered the blood pressure to 160/105 and $T_1$ and $T_2$ became simultaneously positive, showing reversal of the left ventricular strain pattern, which was resumed when the etamon effect wore off.

## DISCUSSION

It is of interest, though not surprising, that hypnotic calm, unlike sleep, has no hypotensive effects. Attempts at co-con-

ditioning with bells rung at the time of relaxing suggestions during and after the hypnotic sessions likewise left the blood pressures unchanged. In a minority of hypnotic sessions, on the other hand, during which we purposely produced emotional storms in the patients by traumatic psychological suggestions, the blood pressure rose up to 20/15 mm. of mercury. Similar rises have been produced experimentally by others, and noted during nightmares of sleep (MacWilliam, 1923). Induced emotional exercise during hypnosis has been accompanied by a rise in venous pressure with no change in arterial or intramuscular pressures (Berg, Delius and Schildger, 1948).

Psychoanalytic therapy has been credited with hypotensive effects in a few hypertensive cases surcharged with repressed hostile and rage impulses (Alexander, 1939). On the other hand, the experience of hypnotherapists like Wolberg (personal communication, 1949) attests to the failure of hypnosis to lower blood pressure. Neither the heart rate nor the respiratory rate decrease in hypnosis as they do in sleep (Jenners and Wible, 1937). The same is true of knee jerk and buzzer reactions (Bass, 1931). The electrocardiographic complexes are unchanged in both sleep and hypnosis (Wible and Jenners, 1936) as opposed to the hypotensive effects of etamon and spinal anesthesia, which frequently reverse the left ventricular strain pattern of hypertensive patients (Ruskin and Lane, 1949).

For these and other reasons, most authorities distinguish clearly between sleep and hypnosis and caution against measuring various physiological reactions by mistake during the sleep which follows deep hypnosis. We guarded against this error by questioning the patient at intervals and terminating the hypnotic sessions before sleep intervened. In 3 instances in which posthypnotic sleep was allowed to ensue, the respiratory rate and pulse rate fell, and the blood pressure fall was similar to those obtained in 3 other cases examined at 10 to 11 P.M., 1 to 2 hours after the onset of natural sleep (Table I). Muscular, nervous and vascular tensions undoubtedly remain at a level above those of sleep during hypnosis, and no amount of relaxing suggestions can lower them to their baselines.

SUMMARY

In seven deeply hypnotized hypertensive patients we failed to influence the blood pressure downward in repeated half-hour sessions, during which relaxing suggestions and post-hypnotic suggestions were constantly and apparently successfully made. Definite hypotensive effects were noted in the same patients following natural sleep, sleep following deep hypnotic trances, and sodium amytal and etamon tests.

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# ERYTHROBLASTS AT BIRTH

JOHN G. SINCLAIR*

Erythroblastosis is generally defined as an abnormally high number of circulating erythroblasts. The number which is normal at birth is estimated quite variously but always very low. In most counts a distinction is not made between normoblasts and definitive erythyroblasts. In some variations of the techniques used the distinction is not evident. The first study of these cells was by Neumann (1874) and the term was coined by Rautmann (1912). The present study covers 1256 consecutive births at John Sealy Hospital, excluding those from which good preparations could not be made for various stated reasons.

The normal course of the red cell change during embryonic and fetal life has been studied in laboratory animals and in man, sometimes from smears and sometimes from sections of fixed embryos aborted alive or recently alive (Wintrobe and Schumacker, 1936). These studies indicate that four or more independent variables are at work (Gilmour, 1941): 1. Cell size becomes diminished, cell boundaries become more circular and refractile. 2. Cell basophilia is highest in primitive hemocytoblasts and diminishes gradually, appearing finally as discrete granular filaments in the reticulocytes. 3. Hemoglobin gradually concentrates, causing acidophilia which is superimposed on the basophilia and results in a definite muddy slate color which gives way to homogeneous clear acidophil staining cytoplasm without granules. 4. The nuclear chromatin becomes condensed and the nuclear diameter is reduced. Any reticulate structure disappears and nucleoli are not separately distinguishable. 5. The nucleus is finally ejected either fragmented or intact, leaving the cell a flat plastid. In general this sequence is followed but cells may eject nuclei of various sizes and degrees of concentration and may do so long before the cytoplasm has attained its full hemoglobin content.

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In order to describe changes in distribution there must be agreement on definitions. Textbooks of hematology are not too clear on this matter and a choice must be made (Wallstein in Downey's Handbook of Hematology, 2, 1938). The following is our choice of terms and characteristics. A new terminology recently suggested is used parenthetically where applicable (Hughes, 1949).

*Reticular cells.* Large irregular cells often with extensive processes. Nuclei 20 or more microns generally spherical or slightly ellipsoid, not very chromatic but reticulate, showing inconspicuous nucleoli. Cytoplasm faintly basophil and may be vacuolate with azurophil granules. They are like giant mesenchymal cells never normally present in circulation. Macrophages and megakaryocytes may be derived directly from this stem cell.

*Hemocytoblasts.* A smaller and more regular cell with 15-micron nucleus. Nucleus more chromatic but reticulate and nucleolate. Cytoplasm quite homogeneous and very basophil. Not a normally circulating cell. Gives rise to myeloblasts and megaloblasts.

*Megaloblast (rubriblast).* A quite spherical cell of 15 microns with a nucleus of about 10 microns. Nucleus still reticulate and nucleolate. Very chromatic and may undergo mitosis. The border zone tends to take acid dyes, indicating various degrees of hemoglobin concentration peripherally.

*Macroblast (prorubricyte).* About the same size as the megaloblast but nucleus reduced to about 7 microns and very dense and refractile. Seldom seen in mitosis. Nucleolus is hardly distinguishable. Some of these cells have large nuclei up to 15 microns nearly as dense as the small ones. Relatively little hemoglobin.

*Definitive erythroblasts (metarubricytes).* The nucleus remains 5–10 microns in diameter and the spherical cell contains nearly complete hemoglobin. The color is still definitely different from the mature cell and is not completely homogeneous. Nuclei in all stages of extrusion.

*Reticulocytes.* Definitive erythroblasts without nuclei.

*Normoblasts.* Nucleus exceedingly dense and reduced to 2–3 microns in a cell of about 7 microns, hemogeneous and fully hemoglobinized.

On the basis of these definitions, the normal distributions during the course of growth are as follows: The reticular cell is never normally in the circulation nor is the macrophage nor megakaryocyte derived from it. The hemocytoblast is also confined to blood producing tissues. The megaloblasts are the common circulating cells of the embryo and may be found in mitosis in the circulation up to approximately the 50 mm. stage or third month. They are reduced to 10 per cent of the red cells by this time and soon disappear entirely. Other nucleated cells of the macroblast and definitive erythroblast types replace them so that up to this stage there is little change in the absolute number per cubic millimeter. The number of cells per cu. mm. in circulation has increased about three times so the percentage of nucleated cells drops. The rate of further decrease of nucleated red cells is not in agreement among authors. Some see none at birth while others find that 92–100 per cent of newborn have nucleated cells. Lucas, Dearing, Hoobler et al. (1922), said that 52 per cent of newborn infants showed some nucleated cells with an average equal to one per cent of the leucocyte count and reduced in 24 hours to half that ratio.

Kato (1932) found that the reticulocyte count also dropped very rapidly after birth. If the cord is tied prematurely, however, the red cell count does not reach the normal high levels, rapid regeneration from narrow becomes prolonged and the reticulocyte count stays up.

## Materials and Methods

In order to have our blood counts representative of condition at birth we first attempted to have internes and nurses make the smears directly from the cords during delivery. The smears were very unsatisfactory because of variations in thickness, failure to dry quickly, greasy slides, and finally adventitious cells from the surface of the cord or jelly of the cord. We then placed a refrigerator in the hall directly opposite the door of the delivery room. Placentas were placed here immediately

and were collected twice daily. Smears were made from superficial veins of the cord or chorionic surface. We found it possible to make maternal blood counts from the uncoagulated blood in sinuses on the other side but did not pursue this aspect of the problem. Smears were stained with Wright's stain and carefully blotted with filter paper. Blotting is an important matter in differentiating degrees of cytoplasmic acidophilia and also in distinguishing the finest detail of granules in leucocytes. Cytoplasmic contrasts are clear cut and leave no doubt. Two smears were made from each placenta and counts of 200 leucocytes plus the corresponding erythroblasts were reduced to percentages. The technique was carried out over a two-year interval, using four technicians at different periods. Their work was found comparable and consistent. Any unusual finding was checked at once.

## Results

The chart (Fig. 1) shows the distribution of nucleated red cells per 100 leucocytes for 1140 full term infants. It is evident that we are dealing with a multimodal curve with at least three modes. Only nine per cent of infants at birth were found free of nucleated red cells. All of these have about 10 per cent of their red cells in the reticulocyte group. The first group with its mode at 0 includes individuals to the 8 per cent group. A new mode appears at 10 per cent with a slope downward to 15–17 per cent. The next mode is near 20–21 per cent and is much less differentiated. The number of cases in any class beyond this is too small to show significant grouping. Approximately half the total have 10 per cent or less nucleated cells. The maximum ranges up to 82 per cent. Those with high counts seem to show no disadvantages compared to their more average neighbors. It was noted, however, that whenever congenital anomalies like club-foot or hare-lip appeared the counts were generally high. Javert (1939) made a similar observation.

We compared the distribution of these cells with birth weight, with anesthetic used, with reported cyanosis, with length of labor and found no correlation unless other pathological signs were present. We are not yet in position to make reliable Rh determinations upon which the validity of such

correlations ultimately depend. Sanford (1926) found good correlation between coagulation time and anesthetic used or other factors producing anoxemia. Some factors act across the placenta but Wiener and Silverman (1940), Dockeray and Sachs (1944), and Polayes (1945) find no constant equilibrium between antibody concentration in mother and child.

**DISTRIBUTION OF ERYTHROBLASTS AT BIRTH IN 1140 NORMAL TERM INFANTS. PERCENTAGE COMPARISON WITH 100 LEUCOCYTES**

| Class | No | Class | No | Class | No | Class | No |
|---|---|---|---|---|---|---|---|
| 0-1 | 167 | 20-21 | 43 | 40-41 | 7 | 60-61 | 4 |
| 2-3 | 159 | 22-23 | 21 | 42-43 | 6 | 62-63 | 4 |
| 4-5 | 138 | 24-25 | 15 | 44-45 | 2 | 64-65 | 2 |
| 6-7 | 91 | 26-27 | 13 | 46-47 | 7 | 68-69 | 1 |
| 8-9 | 73 | 28-29 | 12 | 48-49 | 3 | 70-71 | 1 |
| 10-11 | 141 | 30-31 | 13 | 50-51 | 1 | 72-73 | 2 |
| 12-13 | 55 | 32-33 | 14 | 52-53 | 5 | 74-75 | 1 |
| 14-15 | 25 | 34-35 | 8 | 54-55 | 5 | 76-77 | 1 |
| 16-17 | 34 | 36-37 | 7 | 56-57 | 3 | 78-79 | 2 |
| 18-19 | 31 | 38-39 | 7 | 58-59 | 1 | 80-82 | 1 |

FIGURE 1

Among our series were 103 called premature on the basis of weight. We seriously question this criterion although, on the whole, it includes all premature infants plus some fully mature. The table (Fig. 2) compares the percentage of nucleated cells in premature and full term infants at birth. The 2 per cent showing no nucleated cells may be fully mature. The mode of the whole group has shifted upward. Prema-

**DISTRIBUTION OF ERYTHROBLASTS**

| PREMATURE INFANTS TOTAL 103 | | | STILLBIRTHS 10 | | ERYTHROBLASTOSIS FETALIS 3 |
|---|---|---|---|---|---|
| Class | % | vs normal | No. | % | |
| 0-1 | 2 | 9 | 0 | 0 | 1 at 45 % |
| 2-9 | 38 | 50 | 1 | 10 | 1 at 52 % |
| 10-15 | 19 | 25 | 1 | 10 | 1 at 325 % |
| 15-23 | 13 | 12 | 1 | 10 | |
| 24-49 | 20 | 9 | 5 | 50 | |
| 50+ | 11 | 3 | 2 | 20 | |

FIGURE 2

turity is characterized, therefore, by nucleated cell counts of about 20 per cent. Bungeler and Schwartz (1927) noted less difference and Anderson (1941) placed it at 11 per cent.

Ten of the stillbirths in our series were alive close enough to the time of birth to make usuable blood smears. None of them failed to show nucleated cells and the mode is nearly at 35 per cent. Anderson's group of stillbirths is higher, up to 57 per cent.

Three cases of clinical erythroblastosis appeared and the average count also was high (45, 52 and 325 per cent). Anderson (1941) had lower counts.

It seems clear that definite developmental pathology, prematurity and anoxia due to high Rh antibody are associated with high erythroblast counts. The whole picture is not unlike stages in the remission from pernicious anemia (Wintrobe and Shumacker, 1935). If the high counts in apparently normal infants are significant they must correlate with some hidden or internal condition. In spite of the fact that these counts are rapidly reduced after birth we feel that they are not purely adventitious phenomena, otherwise there would be more correlation with anoxia produced during the birth process. We found none that were consistent.

The nuclei of the cells enumerated are typically very condensed and spherical with refractile borders. The presence of megaloblasts or intermediate forms is very rare. Mitosis has not been seen. The majority of the cells are small, with nuclei 5–7 microns in diameter. True normoblasts are equally rare. The difference in cytoplasmic color is distinct. Silvette (1927) states that the distribution of cell diameters (4–10 microns) is the same as in the adult. Our slides indicate a larger proportion of large cells.

## Summary

The distribution of nucleated red cells in 1256 infants brought to birth shows a multimodal curve with modes at zero, ten, twenty and thirty per cent groups, counting the number of nucleated red cells per 100 leucocytes. The multiple modes cannot be explained nor are the classes related to birth weight, to anesthetic used, length of labor, or recorded degree of cya-

nosis. Prematurity and pathological conditions leading to stillbirth or to clinical erythroblastosis fetalis are correlated with a higher average erythroblast count. The nucleated cells are almost exclusively definitive erythroblasts lacking full hemoglobin content. Free intact nuclei and all degrees of extrusion are seen but karyorrhexis is very rare.

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# CHEMOTHERAPY OF BORRELIA ANSERINA INFECTION (SPIROCHETOSIS) IN YOUNG CHICKS*

ARDZROONY A. PACKCHANIAN

Endemics and epidemics of avian spirochetosis have been found to occur in many parts of the world. Sakharoff (1891) was the first to observe the spirochetes in the blood of sick geese in the Caucasus and regard them as pathogenic. He named the microorganisms *Spirochaeta anserina.* Marchoux and Salimbeni (1903) described a similar disease of fowls in South America and demonstrated its transmission by ticks, *Argas persicus.* The occurrence of outbreaks of avian spirochetosis has been reported also from Europe, Africa, India and Australia. These outbreaks have been reviewed by several authors, such as Knowles, Das Gupta and Basu (1932), and Sreenivasan and Sankaranarayan (1945). Recently the disease has been reported from the United States by Hoffman, Jackson and Rucker (1946), and by McNeil, Hinshaw and Kissling (1949).

Uhlenhuth, Gross and Bickel (1907) apparently were the first to study the action of atoxyl in fowl spirochetosis and found this drug of therapeutic value. Dschunkowsky (1911) reported the curative value of atoxyl and salvarsan. Gonder (1914), working with a salvarsan-resistant strain of *Borrelia anserina, (Spirochaeta gallinarum)* and *B. duttoni,* found that the resistance was maintained after the spirochetes had been passed through a number of animals and ticks *(Argas persicus* and *Ornithodorus moubata).* El-Dardiry (1945) summarized the result of drug treatment and found that arsenicals such as salvarsan, myosalvarsan, "spirocid" and atoxyl gave satisfactory results, while arrhenal (sodium methylarsonate) and sulfanilamide, sulfathiazol, sulfapyridine and sulfadiazine were not effective. Nobrega and Bueno (1946) reported the success-

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ful use of penicillin for spirochetosis in adult chickens when given 5,000 units in five doses at 3-hour intervals, but found that a total of 20,000 units per kilogram body weight in very young chicks failed to produce cure. McNeil, Hinshaw and Kissling (1949) found penicillin in a single dose of 10,000 units was sufficient to cure infected birds from 6 weeks to 12 months of age. They report also the curative value of neoarsphenamine and mapharsen, while streptomycin was found ineffective.

The object of the present communication is to give a brief account of 18 chemicals which the writer used in chemotherapy of *Borrelia anserina* infection (spirochetosis) in young chicks. Fifteen of these drugs are used for the first time against this infection.

## Method and Material

*The strain of Borrelia anserina:* The strain of *Borrelia anserina* was isolated during 1948 from an outbreak of spirochetosis in turkeys in California, and was sent to the writer by courtesy of Drs. McNeil and Hinshaw. This strain is maintained in young chicks by syringe passage.

*Experimental animals:* Three- to four-day-old chicks weighing about 50 grams were selected for this study because *B. anserina* (California strain) infection in very young chicks ran an acute course and always terminated fatally within a week. Half-grown chicks and older chickens are not suitable for chemotherapeutic study because *B. anserina* infection is less regular and spontaneous recoveries frequently occur.

Seventy-five three- to four-day-old chicks were inoculated with *Borrelia anserina.* The second or third day following inoculation, after ascertaining that each chick's blood contained about 100 spirochetes per microscopic field, they were subjected to chemotherapeutic studies.

In order to determine whether or not chicks cured by chemotherapy will harbor *Borrelia* in their brains, a few of the "cured" chicks were killed three weeks after their blood was cleared of spirochetes by chemotherapy. The brain was removed from each bird, macerated and suspended in about three volumes of Tyrode's solution. Then 0.5 ml. of the suspension was inoculated intraperitoneally into a young normal bird.

The remainder of the treated chicks were bled from the heart; the blood was defibrinated, and 0.5 ml. of the blood from each "cured" bird was inoculated intraperitoneally into other normal chicks.

TABLE I

*Chemotherapy of Borrelia Anserina Infection (Spirochetosis) in Young Chicks (Chemicals which were ineffective)*

| No. | Chemical | No. of Chicks Used | Treatment and Results: Mg. Dosage per Injection | No. Injections 24 Hrs. Apart | Results |
|---|---|---|---|---|---|
| 1 | Borrelidin | 6 | 0.25 | 2–4 | Dead in 4–7 days |
| 2 | Beta-ethyl gamma-propyl (di-isothiourea) sulfide dihydrochloride | 3 | 2.0 | 3 | Dead in 6 days |
| 3 | 1(2'-Diethylaminoethylamino) 9-thio 10-xanthone hydrochloride (Miracil) | 4 | 2.0 | 2–4 | Dead in 5–7 days |
| 4 | 10-(2-Dimethylaminoethyl) phenothiazine hydrochloride | 4 | 2.0 | 2–4 | Dead in 5–7 days |
| 5 | 4-amino-6-(2'-amino-6'-methylpyrimidyl-4'-amino) quinaldine-1:1'-dimetho salts (Antrycide salt) | 2 | 1.0 | 1–4 | Dead in 4–7 days |
| 6 | 2:7-diamino-phenyl-10-methylphenanthridinium bromide (Dimidium bromide) | 4 | 2.0 | 2–5 | Dead in 5–8 days |
| 7 | 5-nitro-2-furaldehyde semi-carbazone (Furacin) | 1 | 1.0 | 2 | Dead in 5 days |
| 8 | 9-[Beta-(tri-n-Butylcarbinylmethylamino) ethylamino]-7-methoxy-3-chloroacridine. 2HCl | 4 | 2.0 | 2–4 | Dead in 4–7 days |
| 9 | 4-Dimethylaminocyclohexylamino-6-methoxyquinoline. 2HCl | 2 | 2.0 | 5 | Dead in 8 days |
| 10 | 9-(4-Dimethylaminocyclo-hexylamino)-6, 7-dimethoxy-3-nitrocridine. 2HCl | 4 | 2.0 | 2–5 | Dead in 5–9 days |
| 11 | 9-(4-Dimethylaminocyclo-hexylamino)-7-methoxy-3-chloroacridine. 2HCl | 4 | 2.0 | 2–5 | Dead in 5–9 days |
| 12 | 9-(p-Dimethylaminoanilino)-7-methoxy-3-chloroacridine. HCl. | 4 | 2.0 | 3–5 | Dead in 6–8 days |
| 13 | 9-(Beta-Diisobutylaminoiso-propylamino)-7-methoxyl-3-chloroacridine. 2HCl | 2 | 2.0 | 2–4 | Dead in 4–7 days |

*Chemicals used:** The antibiotic, borrelidin, was received from Hoffmann-La Roche, Inc. Chemicals numbers 2, 3, 4 and 17 were prepared by Eli Lilly & Co.; No. 5, by Imperial Chemical Co., Ltd., No. 6 and 14, by Merck & Co.; No. 7 (Furacin), by Eaton Laboratories, Inc.; numbers 8 through 13, by Wm. S. Merrell Co.; mapharsen, by Parke, Davis & Co.; tryparsamide, and penicillin G, by E. R. Squibb & Sons. (See Tables I and II.)

TABLE II

*Chemotherapy of Borrelia Anserina Infection (Spirochetosis) in Young Chicks (Drugs which produced cure)*

| | | Treatment and Results | | | |
|---|---|---|---|---|---|
| No. | Chemical | No. of Chicks Used | Mg. Dosage per Injection | No. Injections 24 Hrs. Apart | Results |
| 14 | Neoarsphenamine | 2 | 1.0 | 2 | Cured |
| 14 | Neoarsphenamine | 1 | 1.0 | 1 | Cured |
| 15 | Mapharsen | 1 | 0.5 | 2 | Cured |
| 15 | Mapharsen | 1 | 1.0 | 1 | Cured |
| 16 | Tryparsamide | 3 | 2.0 | 4–5 | Dead in 6–8 days |
| 16 | Tryparsamide | 4 | 4.0 | 4–5 | Cured |
| 17 | 4-Carbamidophenyl di(1'-carboxyphenyl 2'-thio) arsenite | 4 | 1.0 | 3 | Cured |
| 17 | 4-Carbamidophenyl di(1'-carboxyphenyl 2'-thio) arsenite | 3 | 2.0 | 1 | Cured |
| 18 | Penicillin G (Crystalline sodium salt) | 4 | 50,000 Units | 1 | Cured |

Borrelidin was received in solution in 1 ml. ampules containing 25 mg. of the antibiotic. The crystalline sodium penicillin G was dissolved in salt solution so that 0.5 ml. contained 50,000 units. The other chemicals were dissolved (or suspended) in physiological salt solution in such concentration that 0.5 ml. of the inoculum contained 0.5 mg., 1 mg., 2 mg. or 4 mg. of the chemical. The drug in selected dosage was injected into the breast muscle of each chick (after determining heavy infection in the chick with *Borrelia anserina*). Usually four chicks were used for testing each drug.

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*Microscopic examination:* A small drop of blood from each treated and control chick was examined microscopically by dark field illumination (high dry objective 45X and ocular 15X) 48 hours after inoculation and each day thereafter until the chicks were found dead or cured. Observations of the spirochetes were recorded as "none found," "few," and when they were many, their number was estimated at 100± or 200± per field, and when they were very numerous and in tangled formation, their number was marked 500+.

## Experimental Data

The results of the present study can be summarized as follows (see also Tables I and II):

(a) *Borrelia anserina infection (spirochetosis) in untreated control chicks:* The experimental infection (spirochetosis) in three- to four-day-old chicks ran an acute course. The spirochetes appeared in the circulating blood on the first or second day following inoculation. They were actively motile, and their number increased progressively and rapidly until the blood of the bird literally was swarming with *Borrelia.* The spirochetes were observed in the blood singly, in small or large agglomerations, in tangled formations and masses of granules. No spirillolytic crisis ever was noted in these young chicks. All experimentally infected untreated control birds died within ten days; the minimum duration of the disease was four days, the average, six.

(b) *Chemicals which failed to produce cure:* The following chemicals, in 0.25 mg., 1 mg. or 2 mg. doses (5 to 40 mg. per kilogram of body weight) administered daily from the second day of infection onward, failed to exert any therapeutic or curative effect (see Table I). All chicks died from spirochetosis within 9 days. Most of the chicks were found dead on the fifth or sixth day following the infection (three or four days after the beginning of chemotherapy).

Borrelidin;

Beta-ethyl-gamma-propyl (diisothiourea) sulfide dihydrochloride;

1(2'-Diethylaminoethylamino) 9-thio 10-xanthone hydrochloride (Miracil);

10-(2-Dimethylaminoethyl) phenothiazine hydrochloride;

4-amino-6-(2'-amino-6'-methylpyrimidyl-4'-amino) quinaldine-1:1'-dimetho salts (Antrycide salt);

2:7-diamino-phenyl-10-methyl-phenanthridinium bromide (Dimidium bromide);

5-nitro-2-furaldehyde semicarbazone (Furacin);

9-[beta-(tri-n-Butylcarbinylmethylamino) ethylamino]-7-methoxy-3-chloroacridine.2 HCl;

4-Dimethylaminocyclohexylamino)-6-methoxyquinoline.2 HCl;

9-(4-Dimethylaminocyclohexylamino)-6, 7-dimethoxy-3-nitroacridine.2 HCl;

9-(4-Dimethylaminocyclohexylamino)-7-methoxy-3-chloroacridine.2 HCl;

9-(p-Dimethylaminoanilino)-7-methoxy-3-chloroacridine. HCl;

9-(Beta-diisobutylaminoisopropylamino)-7-methoxyl-3-choroacridine.2 HCl;

(c) *Drugs which proved of therapeutic value:* The following drugs all had curative effect on *Borrelia anserina* infection in chicks (see Table II):

Neoarsphenamine in a single treatment with one milligram was an effective spirochetocidal and produced cure.

Mapharsen in a single dose of 0.5 mg. reduced the number of spirochetes considerably, while the second dose, also of 0.5 mg., cleared the blood of spirochetes. In other experiments, a single 1 mg. dose was curative.

Tryparsamide in 2 mg. daily doses was ineffective against *Borrelia anserina*, although in one case a temporarily suppressive effect was noted, and the experimentally infected chicks died within eight days by spirochetosis. The same drug, in 4 mg. doses, cleared the blood of the chicks of spirochetes after four or five daily injections.

A new compound, 4-Carbamidophenyl di (1'-carboxyphenyl 2'-thio) arsenite, in a single treatment of a 2 mg. dose was

actively spirochetocidal and produced cure. When the dosage was reduced to 1 mg., it was necessary to give three daily injections to produce the same curative effect.

Penicillin G in a single dose of 50,000 units produced cure.

Chicks which were inoculated with the brains and heart blood from birds which had been cured chemotherapeutically failed to show any spirochetosis, indicating that there were no latent spirochetes in the brains of the treated chicks, which apparently were completely cured.

## Discussion

Variable results are obtained in chemotherapy of *Borrelia anserina* infection if larger chicks or adult fowls are used. The claim that chicks once cured by chemotherapeutic means are immune to reinfection probably is due to the age of the birds. Experimental data on hand has demonstrated that when chicks are two weeks old or older, they are not suitable for evaluating the comparative chemotherapeutic effect of a given drug because spontaneous recovery often occurs in the untreated birds.

## Summary

*Borrelia anserina* infection (spirochetosis) in untreated control chicks ran an acute course. All the chicks invariably died within ten days of spirochetosis. Eighteen chemicals were used in studying the chemotherapy of experimental *Borrelia anserina* infection (spirochetosis) in three- to four-day-old chicks; fifteen of these drugs were used for the first time against this infection. Thirteen of the chemicals were found ineffective against the spirochetes in 2 mg. daily doses. All birds treated with these chemicals died within ten days. The following five drugs, *i.e.*, neoarsphenamine, mapharsen, tryparsamide, 4-carbamidophenyl di (1'-carboxyphenyl 2'-thio) arsenite, and penicillin G, were found to be effective spirochetocidals and completely cured the experimental spirochetosis in chicks.

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# ANTIBACTERIAL FACTOR IN TICK EXTRACTS*

LUDWIK ANIGSTEIN, DOROTHY M. WHITNEY, AND DON W. MICKS

In terms of natural history, the successful transmission of an arthropod-borne infection primarily depends on the maintenance of the infectious agent in the biological vector. In many instances the continuity of the infection is preserved throughout generations of the arthropod provided the latter is resistant to the agent. With the exception of the human body louse which invariably succumbs to the infection with *Rickettsia prowazeki*, all other pathogenic rickettsiae are harmless to their arthropod vectors. The resistance of insects, ticks and mites to various bacterial, protozoal and viral agents which are pathogenic to susceptible higher animals further illustrates the significance of the biological phenomenon.

The problem presents itself as to whether the resistance of the tick to the spotted fever rickettsia or of the mosquito to the malaria parasite or to yellow fever virus merely expresses a balanced host-parasite relationship in terms of true immunity, or whether the protective action is possibly due to some metabolic processes of the host.

Apart from being biological vectors of specific pathogenic agents, arthropods as a group harbor a great variety of bacteria (Steinhaus, 1946). Among the factors influencing the type of bacterial flora the feeding habits of the arthropod are of primary importance. In this regard the blood sucking species occupy a singular position as having the fewest bacteria or even showing sterility of their digestive tracts (Steinhaus, 1946). This is all the more striking as the process of piercing the contaminated skin or hide of animals offers ample opportunity for ingestion of bacteria.

The possibility that a bactericidal factor exists in the digestive tract of bloodsucking insects was therefore a plausible as-

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sumption. It was Nuttal (1899) who first demonstrated that the anthrax bacillus died in the stomach of the bedbug within 24 to 28 hours, but it was not until 1925 that Hindle and Duncan investigated the viability of bacteria in the tick *Argas persicus*. On the basis of their experiments in feeding ticks on various bacteria, the authors concluded that the disappearance of the organisms from the digestive tract is due to "lytic and inhibitory principles" produced by the tick. These observations were followed by those of Duncan (1926) who in testing the digestive tract of flies, mosquitoes, bedbugs and soft ticks found the presence of a bactericidal substance in all of them. Intuitively using techniques comparable with modern antimicrobial assays, Duncan found the spore-forming bacilli of the subtilis group most susceptible. As to the nature of the active principle, it differs according to Duncan from lysozyme and exhibits none of the properties of bacteriophage.

The present study developed from our recent inquiry into the prevalence of rickettsial infections in "wild" ticks of the Gulf area and from a bacteriological survey of these ticks. The scarcity or absence of bacterial flora corroborated the findings of previous authors and stimulated us to investigate the underlying causes of this phenomenon.

## Material and Methods

The following ticks were the source of material under study: *Amblyomma americanum*, *A. cajennense*, *Rhipicephalus sanguineus*, *Ornithodoros turicata*, *Argas persicus*. The hard ticks were obtained either by dragging a "flag" over ground vegetation (unfed ticks) or by removing engorged specimens from cattle or dogs. Most of the work was done on blood engorged specimens, including *Argas* collected from chicken houses and *Ornithodoros* supplied by Dr. V. T. Schuchardt, The University of Texas, Austin, from his tick colony (Ticktorium). Live unfed ticks were used in pools of a few dozens to several hundred. These were anesthetized, rinsed thoroughly with sterile saline, ground aseptically with alundum in mortar and, finally, suspended in saline in proportion of 10 ml. to 100 ticks. After centrifugation (1,000 rpm. for 10 min.) the usually sterile supernate was tested for antibacterial activity either directly

or after lyophilization. The live engorged ticks were treated individually by evisceration after dipping them in Zephiran solution (1:1,000) and rinsing with three changes of sterile saline. The bloody, viscous content was ground and extracted in distilled water (1:3 parts by volume). The heavy suspension was then distributed in ampules (3 ml. each) and lyophilized, yielding about 0.1 gm. of the reddish-brown powder per sample. Immediately before use the dry material was resuspended in proportion of 0.1 gm. in 5.0 ml. (1:50) of phosphate buffer (pH 7.8), and heated in water bath of 65°C. for 30 minutes or treated with trypsin at 37°C. for 4 hours and then heated to 65°C. for 30 minutes.

For tests on antibacterial activity the streak plate technique of antibiotic assays was used as follows: agar plates dried in 37°C. for 48 hours were inoculated with *Bacillus subtilis* as test organism (Randall, Price and Welch, 1945) by drawing parallel lines across the surface with a loop carrying a light suspension of spores (1:1,000 dilution in distilled water of a 48 hour agar surface slant culture heated in 65°C. water bath for 30 minutes). These plates were dried in the refrigerator for 48 hours by inverting over sterile filter paper in the top of the Petri dish. Prior to incubation a single drop of tick extract was placed through a 27 gauge needle in two opposite sections of the streak. In addition to 3 strains of *B. subtilis*, the following gram positive test organisms were used: *Bacillus pumilus*, *B. megatherium*, *B. anthracis*, *Sarcina lutea*, *Staphylococcus aureus*, *S. albus*, *Streptococcus pyogenes*, *S. agalacticus* (Table 2).

In using the spot dilution method (Thomas, Levine, Vitagliano, 1944), dried agar plates were flooded with a 1:1,000 suspension of subtilis spores and kept in the refrigerator for 2 days before use. Single drops of serial two-fold dilutions of the tick material were placed clockwise on the agar plates and incubated at room temperature (about 30°C.).

Other assay methods included paper discs, penicylinders and serial dilutions in broth. For digestion of tick tissues trypsin (Pfanstiehl powder, activity 1:110) was used (0.1 ml. of 3% trypsin to each 5.0 ml. tick material in phosphate buffer, pH

7.8). The mixture was then incubated at 37°C. for 4 hours followed by heating to 65°C. for 30 minutes. Consequently, an extrinsic proteolytic enzyme was added to the material already partially digested by the tick.

## Experiments and Results

*Qualitative tests.* The most prevalent tick in the Gulf area, *Amblyomma americanum*, served as object for the preliminary tests. The first indication of antibacterial properties was obtained from unfed ticks dragged from a heavily infested area. A fresh extract from 169 adult specimens (great majority were females) in 20 ml. nutrient broth was passed through a fritted glass filter (grade U. F.). The bacteriologically sterile filtrate was pipetted into penicylinders (0.3 ml. each) on an agar plate previously flooded with a *Bacillus subtilis* culture. After 18 hours incubation at room temperature each cylinder was found in the center of a clear, circular area of 1 to 1¼ inches in diameter, walled off from a heavy growth of *B. subtilis*. Further incubation at 37°C. for 6 hours intensified the growth, sharply delineating the inhibition zones.

Having established that unfed ticks are provided with antibacterial properties, further studies were made on various genera and species of ticks either unfed or engorged with blood of their respective hosts. Lyophilized material was regularly used and found convenient for uniformity of procedures and for preservation. The following hard ticks were included: *A. americanum*, *A. cajennense*, *Rhipicephalus sanguineus*. In this series the unfed *Rhipicephalus* showed no antibacterial activity, but a significant inhibition of *B. subtilis* growth occurred in all other instances as revealed by the agar plate streak method. Lyophilized material from unfed and trypsin treated *A. americanum* and *A. cajennense* showed partial or complete inhibition of growth in the contact areas. The same results were achieved with the digested portion of engorged *R. sanguineus* extract while the non-digested part of the same material was less active. The antibacterial activity of *Ornithodoros turicata* was of high intensity as demonstrated by agar plate streaks, paper discs or penicylinders. Extensive, confluent inhibition zones surrounding the cylinders containing digested

material from engorged ticks were striking although a well developed bacteria-free zone was also produced by unfed *Ornithodoros*. The paper disc method seems in this case less sensitive, probably due to a low diffusion rate of the material (Plate 1).

Another representative of soft ticks used in this study was *Argas persicus* collected from chicken houses. Material from fully engorged ticks in a series of parallel experiments before and after trypsin digestion was tested by agar plate streaks. Clear, bacteria-free spaces resulted from contact with the digested tick material, whereas no activity was shown by the non-digested portion.

Very little is known about the digestive enzymes of ticks, but we can presume that they resemble those of insects whose enzymes are adapted to their particular diet (Wigglesworth, 1947). We may therefore anticipate that in ticks feeding exclusively on blood protease is produced in abundance. According to Wigglesworth, protease of blood sucking insects is made up of proteinase acting upon natural proteins, and of peptidases by which the products of protein digestion are further hydrolysed. Since the proteinase is always of the tryptic type, the addition of the homologous enzyme in our experiments merely led to the continuation of enzymatic hydrolysis of the tick content, liberating polypeptides and perhaps

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PLATE I

Inhibitory effect of extracts from *Ornithodoros turicata* on growth of *Bacillus subtilis*.

Plates 1, 2, 3, 4: Upper streaks treated with extracts from *unfed* ticks. Lower streaks treated with extracts from blood-*engorged* ticks.

Plates 1 and 2: Trypsin digested extract used.

Plates 3 and 4: No trypsin used.

Plate 5: Paper disc assay. a. Antibiotic Q 19 used for comparison; b. Extract from *unfed* ticks after tryptic digestion. No growth inhibition; c. & d. Extracts from *engorged* ticks after tryptic digestion. Clear inhibition areas surrounding the discs.

Plate 6: Penicylinder assay. e. Antibiotic Q 19 used for comparison; f. Extract from *unfed* ticks treated with trypsin. Well developed inhibition zone. g. & h. Extract from *engorged* ticks after tryptic digestion showing large, confluent zones of growth inhibition.

1
2
3
4
5
6
e
f
g
h

some free amino acids. It therefore would be logical to assume that the chemical nature of the active principle in the tick lies within the range of these physiological products.

Since our interest was mainly directed to the antibacterial factor inherent in the tick, parallel experiments on fasting and engorged specimens were conducted whenever possible. For this purpose extracts were prepared from flat unfed specimens of *Argas persicus* kept alive in the refrigerator over a period of 2 months and then tested for antibacterial activity. Parallel tests were conducted with another series of *A. persicus* artificially engorged with chicken blood and kept in the refrigerator for 6 days before the test. The presence of the antibacterial factor previously found by us in the unfed *Amblyomma americanum* was supplemented by the establishment of partial or complete growth inhibition of all tested organisms except *Staphylococcus aureus* by extracts from unfed *Argas persicus* (Table 2). However a definite and almost constant increase in intensity of the phenomenon was shown by the series of engorged *Argas persicus*, the latter being of exceptionally high antibacterial potency (titer 1:25,600; Table 1).

As compared with extracts of hard ticks, the range of activity of *A. persicus* on the gram positive organisms tested includes *Bacillus anthracis* and to a lesser extent *Staphylococcus aureus* both of which were unaffected by engorged *A. americanum* and *R. sanguineus* (Table 2). Preliminary tests with some gram negative bacteria of the typhoid-colon group gave no indication of growth inhibition with either of the tick extracts.

*Quantitative tests.* For further evaluation of antibacterial activity of various ticks two-fold serial dilutions of crude extracts in nutrient broth were prepared and tested against one drop of *B. subtilis* spore suspension (2 million spores in 1 ml.). The tick material (20 mg. dehydrated extract in 1.0 ml. broth) was divided in two portions, one digested with trypsin and the other untreated. Tubes showing no visible growth after 24 hours incubation at 37°C. were considered as end points in the series of decreasing concentrations. As seen from Table 1, the uniformly higher activity of the digested portion of extracts either from engorged or unfed ticks against the sensitive

TABLE 1

*Quantitative Evaluation of Antibacterial Activity of Various Ticks Against B. subtilis*
Two-fold dilutions of tick extracts in broth. Endpoints in decreasing concentrations.

| Material | 1:50 | 1:100 | 1:200 | 1:400 | 1:800 | 1:1600 | 1:3200 | 1:6400 | 1:12800 | 1:25600 |
|---|---|---|---|---|---|---|---|---|---|---|
| Amblyomma american. engorged & digested | + | + | + | + | + | + | + | + | — | — |
| Same, not digested | + | + | + | + | + | + | — | — | — | — |
| A. cajennense engorged & digested | + | + | + | + | + | + | + | — | — | — |
| Same, not digested | + | + | + | + | + | + | — | — | — | — |
| Rhipicephalus sanguineus, engored & dig. | + | + | + | + | + | + | + | + | — | — |
| Same, not digested | + | + | + | + | + | — | — | — | — | — |
| Ornithodoros turicata engorged & digested | + | + | + | + | + | + | + | + | + | — |
| Same, not digested | + | + | + | + | + | + | + | — | — | — |
| O. turicata, unfed & digested | + | + | + | + | + | + | — | — | — | — |
| Same, not digested | + | + | + | + | + | — | — | — | — | — |
| Argas persicus engorged & digested | + | + | + | + | + | + | + | + | + | + |
| Trypsin alone in broth incubated at 37°C. | — | — | — | — | — | — | — | — | — | — |

Legend: + complete inhibition; no visible growth of *B. subtilis.*
— no inhibition, normal growth of *B. subtilis.*
First test tube contains 10 mg. dehydrated tick extract in 0.5 ml. broth.
Readings after 24 hours incubation at 37°C.

*B. subtilis* holds true for all the hard and soft ticks tested and can be evaluated by a "titer." However the latter may vary with the individual batches of ticks and can not be considered as a characteristic attribute of a given species.

TABLE 2

*Inhibition of Growth of Various Organisms by Crude Tick Extracts*

| Test Organism | Argas persicus unfed | Argas persicus engorged | Amblyomma americanum engorged | Rhipicephalus sanguineus engorged |
|---|---|---|---|---|
| Bacillus subtilis (Warburg, S.) | ± | + | + | + |
| B. subtilis (Ford, R.) | ± | + | + | + |
| B. subtilis (Lab.) | ± | + | + | + |
| B. pumilus | ± | + | ± | + |
| B. megatherium | + | + | + | + |
| B. anthracis | ± | + | — | — |
| Sarcina lutea | ± | + | ± | + |
| Staphylococcus aureus | — | ± | — | — |
| Staphylococcus albus | ± | ± | ± | ± |
| Streptococcus pyogenes | ? | + | + | + |
| Streptococcus agalacticus | ? | + | + | ± |

Legend: + complete inhibition; ± partial inhibition; — no inhibition; Lyophilized and trypsin digested material from unfed or engorged ticks at ph 7.8; readings after 18 hours at room T., followed by 6 hours in 37°C. Streak plate method.

For the quantitative spot dilution method (Thomas, Levine and Vigliano, 1944) trypsin digested tick material adjusted to pH 7.8 was tested in concentrations from 1:50 to 1:3200. Uniform, single drops of the series were placed clockwise in a circle on agar plates previously flooded with subtilis spores. Readings were made after 20 or 24 hours incubation at room temperature. In the series of *Amblyomma americanum* complete growth inhibition as shown by clear bacteria-free "punched out" circular spots was recorded in the four concentrations (1:50 to 1:400). Agar plugs from the center of the clear areas were placed in nutrient broth and incubated for 24 hours at 37°C. with the resulting sterility of the medium.

*Some Properties of the Antibacterial Factor*

It has been shown that lyophilization to a dry state of material kept in air-filled sealed ampules preserves its activity for

at least 6 months in the refrigerator. This corroborates Duncan's observations on the durability of the active principle.

The resistance to higher temperatures is shown by the routine tests which requires heating to 60–65°C. Furthermore, in connection with the trypsin digestion of the tick extract the latter was occasionally boiled in a water bath for 10 minutes with retention of its antibacterial activity. Finally, autoclaving was applied in using lyophilized and digested extracts from engorged *Ornithodoros* in tryptose broth (pH 7.0) at 115°C. for 15 minutes. A duplicate control series was left unheated. The quantitative tests for activity gave the following results: the unheated sample inhibited the growth of *B. subtilis* in dilutions up to 1:1600, whereas the titer of the autoclaved material was reduced to 1:200.

The themostability and activity of most of the antibiotics are correlated with the pH of the environment. It was thought that a similar association may exist in this instance. For this inquiry a series of samples from *A. americanum* (engorged, digested, lyophilized) was prepared and the suspensions adjusted in phosphate buffer to the following pH values: 4.0, 5.7; 7.0; 8.0. These were autoclaved at 121°C. for 15 minutes. After this procedure a complete inhibition of growth was recorded by the agar plate streak method at pH 7.0 and 8.0, whereas no antibacterial activity was observed with samples of pH 5.7 and 4.0. Quantitative tests by the spot dilution method revealed that although autoclaving destroyed completely or partially the activity of the substance at pH 5.7, the active principle survived autoclaving at pH 7.8 up to 9.4 although to a lesser degree in the latter. The unchanged appearance of the trypsin digested material after autoclaving at pH 8.0 contrasted with the heavy precipitate in similarly treated tick suspension of pH 5.5. No antibacterial activity was found in the supernate of the latter sample. It is therefore assumed that the active principle was most probably carried down with the precipitate or inactivated by heat in acid medium. No change of activity was noted when the extract at pH 5.5 remained in room temperature for one hour.

*Inhibitory Action of Blood*

The high activity of the material from engorged ticks suggested that the blood may play an additional role in the growth inhibition. For preliminary tests guinea pig blood was used inasmuch as in the series of experiments this animal served as feeder of *Amblyomma americanum*. The blood was divided in two main portions: one was left coagulated, while the other was defibrinated mechanically, and centrifuged. Serum was separated from the cells which were washed in sterile saline. Each component was lyophilized and either digested with trypsin or left untreated. The same technique was adopted as applied to the tick material in the final testing of blood samples. A total of 10 blood samples from guinea pigs was examined in various combinations (Table 3).

TABLE 3

*Inhibition of B. Subtilis Growth by Normal Guinea Pig Blood*

| No. Blood sample | Inhibition |
|---|---|
| 1. Whole blood in saline | — |
| 2. Whole blood in buffer ph 7.8 | ± |
| 3. Whole blood in buffer and trypsin | + |
| 4. Washed cells in saline | — |
| 5. Washed cells in buffer | — |
| 6. Washed cells in buffer and trypsin | + |
| 7. Serum in saline | — |
| 8. Serum in buffer | — |
| 9. Serum in buffer and trypsin | — |
| 10. Trypsin in buffer | — |

Blood and serum used in dilutions 1:10. All samples except Nos. 1, 4, 7, heated to 65°C. for 30 minutes.

As seen from the results a complete inhibition of *B. subtilis* growth was achieved on agar plate streaks when trypsin digested cells were used with only partial inhibition with whole digested blood. It is possible that blood coagulation after heating to 65°C. limited the antibacterial action of the latter. The complete absence of antibacterial activity of the serum is of interest since it is known to contain non-specific thermostable substances such as beta-lysins (Topley and Wislon, 1946, p. 1173).

The presence of *B. subtilis* inhibiting substances in normal human sera was recently recorded in connection with penicillin assays, however the active principle was found thermolabile since inactivated sera either lost completely or partially their inhibitory properties (Bugg, Bronstein, et al., 1946).

The antibacterial properties of normal blood were demonstrated by us only after tryptic digestion which fact leads to the assumption that the antibacterial factor (or factors) is correlated with enzymatic hydrolysis ending with peptides or amino acids.*

Since the antibacterial factor found in ticks is considerably enhanced by the ingested mammalian blood, the question arises whether this phenomenon may have some bearing on the "reactivation" of spotted fever rickettsiae in nature as formulated by Spencer and Parker (1923). In reviving this almost forgotten theory, the ingestion of fresh blood is, according to these authors, essential to transform the non-virulent phase of the "virus" of the unfed infected ticks to a virulent fever-producing phase. Some unrecognized condition initiated by the ingestion of blood was assumed to play a part in this transition, which may be due to metabolic processes of the vector, inasmuch as the tick *Dermacentor andersoni* requires up to 48 hours of attachment to the host to become infective. In the light of our observations this "unrecognized condition" might be caused by the enzymatic hydrolysis of blood proteins creating in the tick conditions unfavorable to bacteria, but suitable to the different metabolic requirements of parasitic rickettsiae.

## Summary and Conclusions

The relative scarcity of bacterial flora in blood sucking arthropods and their resistance to specific pathogenic agents were the significant phenomena which stimulated the present

*Independently, and at our request, the chemical nature of a sample of dessicated material from engorged *A. americanum* has been investigated by Dr. Jorge Awapara of The University of Texas M. D. Anderson Cancer Research Hospital, Houston. The results of this work suggested that there were very few amino acids and only one peptide was detected in the crude tick extract. This peptide (Y) stated by Dr. Awapara as similar to or identical with one found by him in human blood exhibits growth inhibition of *B. subtilis* similarly to the tick extracts (personal communication).

study. Furthermore, previous observations by Hindle and Duncan (1925) and by Duncan (1926) on a bactericidal principle in insects and ticks were particularly inducing to analyze this phenomenon.

Hard and soft ticks of Texas *(Amblyomma americanum, A. cajennense, Rhipicephalus sanguineus, Ornithodoros turicata, Argas persicus)* in their adult stage were investigated in the search for antibacterial properties. Parallel experiments were conducted with unfed, fasting ticks and with blood-engorged specimens, the latter feeding on cattle, guinea pigs, dogs, rats and chickens.

For preliminary antimicrobial assays *Bacillus subtilis* was chosen as representative test organism. The following gram positive bacteria were included in the tests and found sensitive to the action of tick extracts: *Bacillus pumilus, B. megatherium, B. anthracis, Sarcina lutea, Staphylococcus aureus, S. albus, Streptococcus pyogenes, S. agalacticus.* Aqueous tick extracts were reconstituted from previously lyophilized material on the basis of 0.1 gm. dry weight of pooled ticks in 5.0 ml. of the liquid (1:50). This was followed by digestion with trypsin which proved essential in handling the high protein content of engorged ticks and for the liberation of the active factor. Parallel tests for antibacterial activity were conducted with the digested and non-digested portions of the material. The following antimicrobial technique was applied: streak agar plates, paper discs, penicylinders, serial broth dilutions and spot dilutions on agar plates. In areas where the extract of various ticks contacted the streaks of the above listed organisms, a complete or partial growth inhibition was revealed after incubation. This phenomenon was constantly observed when engorged ticks were used and to a lesser extent in unfed ticks. Tryptic digestion of the tick material exerted an enhancing influence on the antibacterial factor inherent in the tick and intensified by the ingested mammalian blood. Several properties of the antibacterial factor have been determined: it resists dehydration *in vacuo* and remains active in a dry state when kept in air filled sealed ampules for at least 6 months under refrigeration. It resists heat to 121°C. for 15 minutes in liquid medium at pH 7.8 to 9.4, but will lose its antibacterial activity

at pH 5.7 or lower. Since the antibacterial factor present in the tick is not only resistant to tryptic digestion but is intensified by proteolysis, it is assumed that the phenomenon is correlated with peptides or amino acids.

When normal guinea pig blood was treated by the same technique as tick material, a similar antibacterial effect was demonstrated. This phenomenon was limited to the tryptic digested cells, the non-treated cells showing no activity against *B. subtilis*. Serum samples were entirely inactive.

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# FALL MEETING, TEXAS BRANCH, SOCIETY OF AMERICAN BACTERIOLOGISTS

NOVEMBER 19, 1949

THE UNIVERSITY OF TEXAS, AUSTIN

PROGRAM

The Occurrence and Importance of the Genus *Pseudomonas* in "Infectious" Types of Asthma.

C. D. Dukes and A. M. Lonergan, Allergy Research Foundation and Department of Bacteriology, Baylor College of Dentistry, Dallas.

Activation and Titration of Serum Protease—A Positive Approach Toward Analysis of the Mechanism of Allergy.

Kenneth L. Burden, Rosemary F. Mudd and Georgia Weller, Department of Microbiology, Baylor University, College of Medicine, Houston.

Brucella Survey Amongst Packing House Workers.

Shirley Hagans and A. B. Rich, City Board of Health Laboratories, San Antonio.

Recovery of Virus Paralyzing Baby Mice from Stool of Poliomyelitis Suspect.

Joyce W. Rowen, Margaret Ann Neville and J. V. Irons, Texas State Department of Health, Austin.

A Virus Pathogenic for Suckling Mice Isolated from Patients With a Disease Resembling Poliomyelitis.

S. Edward Sulkin, G. P. Manire and Thomas W. Farmer, Department of Bacteriology and Neurology, Southwestern Medical School, The University of Texas, Dallas.

Epidemiology and Laboratory Findings in a Familial Outbreak of Poliomyelitis.

Dorothy Shelton, Ralph O. Anslow and Dwight M. Kuhns, Fourth Army Area Medical Laboratory, Brooke Army Medical Center, Fort Sam Houston.

A Field and Laboratory Investigation of an Outbreak of Ornithosis (Psittacosis).

J. V. Irons and Thelma D. Sullivan, Texas State Department of Health, Austin.

Toxin Neutralization Studies on the Psittacosis Viruses.

G. P. Manire and K. F. Meyer, Department of Bacteriology, Southwestern Medical School of The University of Texas, Dallas, and the George Williams Hooper Foundation, University of California Medical Center, San Francisco.

Laboratory Findings on Rabies in Texas Wildlife.

Margaret Ann Neville and Thelma D. Sullivan, Texas State Department of Health, Austin.

Susceptibility of Pathogenic Fungi to Mycomycin.

Edwin A. Johnson, Baylor University, College of Medicine, Houston.

Chemotherapy of Typhoid Fever, Clinical and Laboratory Findings.

Elise H. Groos, Joseph H. Cannon, Albert Leibovitz and Dwight M. Kuhns, Fourth Army Area Medical Laboratory and Brooke General Hospital, Fort Sam Houston.

Tissue Culture Studies of Human Ciliated Nasal Mucosa.

J. M. Rose and C. M. Pomerat, Tissue Culture Laboratory, The University of Texas, Galveston.

Mass Mortality of Fish Associated with the Protozoan *Gonyaulex* in the Gulf of Mexico.

Cecil H. Connell and Joy Barnes Cross, Department of Preventive Medicine and Public Health, The University of Texas, Galveston.

Antibacterial Activity of a Substance Present in Ticks (Ixodoidea) of Texas.

Part I. Ludwik Anigstein, Dorothy M. Whitney and Don W. Micks, Rickettsial Research Laboratory, The University of Texas, Galveston.

I. M. Lewis Lecture.

Speaker: Dr. J. L. Etchells.

Subject: Salt-Tolerant Yeasts from Commercial Cucumber Brines.

ABSTRACTS

Salt-Tolerant Yeasts from Commercial Cucumber Brines. John L. Etchells, Bureau of Agricultural and Industrial Chemistry, United States Department of Agriculture, Raleigh, North Carolina.

I. *Subsurface or Fermentative Types*

A part of the microbiological activity of commercial cucumber brines is due to yeasts. Although population studies have been made for several years, no attempt has been made to classify the predominating types. During the 1946 and 1947 seasons, 1,444 isolates were obtained by frequent sampling of 42 fermenting vats at two commercial pickle plants in Eastern North Carolina. During the period in which isolations were made, the brine strength of all fermentations was about 10 to 18 per cent by weight. The isolates were reduced to the following six genera in the order of frequency of isolation: *Torulopsis* (721): *Brettanomyces* (588); *Zygosaccharomyces* (59); *Hansenula* (49); *Torulaspora* (6); *Kloeckera* (1); and 20 isolates not fully classified. The first two genera named represented a total of 1,309 cultures, or slightly over 90 per cent of all yeasts isolated. Furthermore, two new species within these same genera, the tiny yeast *Torulopsis caroliniana nov. sp.* (717), and the very fermentative yeast, *Brettanomyces versatilis nov. sp.* (561), predominated, accounting for 1,278, or slightly over 88 per cent of the total cultures. The first species dominated the early part of the 42 fermentations studied and was followed by the second in a slow fermentation which continued up to the end of the observation period (about 100 days). Twenty-seven isolates of a second new species of *Brettanomyces* named *B. sphaericus nov. sp.* were also obtained during the latter stage of fermentation. The remaining four *Torulopsis* isolates were found to be *T. holmii*. The less frequently isolated genera *(Zygosaccharomyces, Hansenula, Torulaspora* and *Kloeckera)*, consisting of 115 cultures, were placed as follows: 59 *Zygosaccharomyces*, 50 type A, 9 type B; 49 *Hansenula subpelliculosa;* 6 *Torulaspora rosei;* and 1 *Kloechera magna.* The presence of the *Brettanomyces* in such numbers is significant,

since the occurrence of this genus has not previously been described in this country.

II. *Surface or Oxidative Types*

Cucumber brines, sheltered from direct sunlight, develop luxuriant surface growth attributed to film-forming yeasts. A collection was made of 47 film yeasts, representing five brining areas in the country. These cultures came from the surface films of brines ranging in salt content from 5 to 19 per cent by weight and represented films on 40 commercial vat brines in the following geographical areas: North Carolina, 14 vats; Wisconsin, 12; Indiana, 6; Georgia, 5; and Michigan, 3. The 47 cultures were placed in the following genera: *Debaryomyces*, 22 isolates, *Endomycopsis*, 12; *Zygosaccharomyces*, 9; and *Candida*, 4. An additional 14 isolates of *Endomycopsis* were obtained from a like number of films on cucumbers brined under laboratory conditions and were a different variety from those on commercial brines. Yeasts belonging to the genus *Debaryomyces* were the most widespread and were found on brines in all five states. Further, the genera *Zygosaccharomyces*, *Endomycopsis* and *Candida* have not been considered associated with film formation on cucumber brines or other types of food brines. Representative isolates of the genera *Debaryomyces* and *Endomycopsis* were capable of de-esterifying citrus pectin in a cultural medium.

Some Practical Clinical Aspects of Antibiotic Sensitivity by the Disc Method. Anthony A. Contreras, Conan H. Millstein, and Hinton J. Baker, Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston.

The plate disc method for determination of antibiotic sensitivity of organisms from wound cultures was reviewed, and evaluation of commercially prepared discs for antibiotic sensitivity was presented.

The advantages of the commercially prepared disc are:

(1) Additional saving of materials and personnel time.
(2) Antibiotics in dry discs should keep potency for longer periods.
(3) Commercially prepared discs are dyed to aid in identification of each of the antibiotics incorporated.

Two clinical cases were presented to demonstrate the advantages of rapid results of the plact disc method for antibiotic sensitivity.

Effect of Streptomycin on Growth of Certain Fungi. John B. Loefer, Frederick W. Bieberdorf and Russell G. Weichlein, Foundation of Applied Research, San Antonio.

Streptomycin was tested in concentrations ranging from 0.1–10.0 milligrams per milliliter on growth of sixteen species of fungi. After an incubation period of several weeks, either inhibition or acceleration, in direct proportion to the amount of streptomycin present, was obtained for the following species: *Nocardia sp.*, *Candida albicans*, *Cryptococcus neoformans*, *Coccidiodes immitis*, *Sporotrichum schenckii*, *Trichophyton mentagrophytes*, and *Penicillium ochraceum*.

Preliminary results indicate that growth and response of some species appears to be dependent on certain factors, *e.g.*, presence of peptone in the medium, hydrogen-ion concentration or the temperature at which cultures are incubated. Growth of *Candida albicans* in a peptone medium at pH 6.0, for example, was inhibited by streptomycin, but at the same pH growth was progressively accelerated when this substance was added to an inorganic medium. Similar growth was observed with *Cryptococcus neoformans* and *Nocardia* sp. in the two types of media. It is possible that seemingly conflicting results on effect of streptomycin on fungi reported in the literature may have been due to similar differences in environmental factors. The normal yellow color which is characteristic of *Nocardia* cultures was absent when the medium contained 2.5–10 milligrams of streptomycin per milliliter.

Bacterial Utilization of Anionic Surface Active Agents. H. B. Rees, Jr., and O. B. Williams, Department of Bacteriology, The University of Texas, Austin, Texas.

Chance observation of bacterial-like growth in a bottle of commercial detergent shampoo led to culturing and recovery of a *Pseudomonas* sp. It was capable of utilizing the shampoo or sodium lauryl sulfat as a *C* source for growth on solid media with nitrogen supplied as ammonium ion. Other organisms found to utilize the same detergents for growth were *Pseudo-*

*monas fluorescens* and *P. aeruginosa:* those using only shampoo were *Proteus vulgaris, P. morgani, Echerichia coli, Aerobacter aerogenes,* and *Serratia marcescens.*

Cysteine Toxicity for *Brucella abortus* 1257. V. T. Schuhardt, L. J. Rode, and Glenda Oglesby, Brucellosis Research Project of the Clayton Foundation and Department of Bacteriology, The University of Texas, Austin.

When varying concentrations of cysteine in McCullough and Dick mineral base medium were inoculated with small numbers of *B. abortus* 1257 a zone of toxicity was observed between concentrations of 3–6 and 100–200 micrograms of cysteine per ml. of medium. Lower and higher concentrations of cysteine permitted growth of the inoculum used. In parallel tests cystine proved toxic at all concentrations above 3 micrograms per ml. We believe that the toxicity of the cysteine was due to its partial oxidation to cystine. The non-toxicity of high concentrations of cysteine, we believe, is due to a reversal of the cystine toxicity by residual unoxidized cysteine. The addition of high concentrations of cysteine to toxic concentrations of cystine reversed the cystine toxicity. Also the addition of small concentrations of the organic reducing agent sodium-formaldehyde-sulfoxylate to varying concentrations of cysteine tended to eliminate the toxic zone of the latter for *B. abortus* 1257.

The Cultivation of Endamoeba Histolytica in a Non-Fermentable Solid Media. Alberdena Ruysenaars, The University of Texas Medical Branch, Galveston.

The usual Bacto-endamoeba medium of Difco for the cultivation of *Endamoeba histolytica* contains a substance which is fermented by the Endamoeba symbionts. This results in the formation of large gas bubbles and splitting of the agar and permits the amoeba to slide out of reach making recovery difficult. In some instances the gas formation forces the amoeba up beyond the serum-saline overlay and interferes with their viability.

In an effort to eliminate this difficulty a medium similar to the above but with the fermentable substance first removed by a yeast in one case and by *E. coli* in another was tested for a diminution in gas production by the associated bacteria.

Results show a decrease in gas production of 82.7% in the yeast and 91.4% in the *E. coli* prefermented media. The prefermentation of the liver infusion does not affect growth thereon of the endamoeba, and they remained viable for longer periods of time without repeated subculture.

Hemolytic Aerobic Actinomycetes. Frederick W. Bieberdorf, Foundation of Applied Research, San Antonio, Texas.

An investigation as to the hemolytic properties of various species and strains of aerobic actinomyces including two strains *Nocardia asteroides* was made. The purpose of this investigation was to determine if the hemolytic property of these microorganisms is consistent and if this phenomena might be useful as a basis for pathogenicity diagnosis.

The various organisms used in this study were obtained from bronchial exudates, brain lesion (autopsy), facial and skin lesions of patients, airborne contaminants, animal exudate and from a type culture collection. A total of 16 forms was studied. These microorganisms were grown on fresh sheep blood media. Ten of the 16 forms showed some hemolysis. Six of these hemolyzed an area of 3 or more cm. in diameter at ten days. Studies were made at both 37°C. and room temperature with little, if any, apparent difference in their hemolytic properties. Repeated transfers indicated some loss of this phenomena. All of the organisms grew readily on blood agar and triptose agar.

Rigdon, R. H. and Breslin, Dorothy (Laboratory of Experimental Pathology, The University of Texas Medical Branch, Galveston, Texas). Effect of Human Blood on *Plasmodium Knowlesi* Infection in Monkeys.

Human blood when given intravenously and intraperitoneally to monkeys infected with *P. knowlesi* produces a marked diminution in the number of parasitized red cells within the circulating blood. Human plasma and hemoglobin does not effect the degree of parasitemia. The mechanism of this effect on the parasitemia is not known.

*M. mulatta* monkeys rarely show any evidence of sensitivity to human blood—only one in a group of 25. Furthermore, these monkeys did not develop sensitivity to human blood after multiple injections of either blood or plasma.

# TISSUE CULTURES OF ADULT HUMAN CEREBRAL CORTEX*

C. M. Pomerat, J. R. Ewalt, S. R. Snodgrass and M. F. Orr

Embryonic nervous tissue has long been a favorite material employed by workers using tissue culture technique for the demonstration of nerve fibers and glial elements. Recently Hogue (1947) described the outwandering of Purkinje cells from explants of the cerebellum of human embryos. While adult tissues are generally more difficult to cultivate, Murray and Stout (1947) have succeeded in obtaining migration of nerve cells from human sympathetic ganglia, with definite evidence of mitotic activity. In a series of classic papers, Russell and Bland (1933, 1934), and Canti, Bland, and Russell (1937) demonstrated that a wide variety of brain tumors could be cultivated *in vitro*. However, these studies dealt with outgrowth of malignant elements, with little emphasis on other cell types.

The present study is concerned with biopsies of adult human cerebral cortex which were obtained from 55 schizophrenic patients subjected to prefrontal lobotomy. Tissues were placed in Gey's solution and within one hour from the time of operation they were cut into fragments approximately 2 mm. square and 1 mm. thick. The most successful method of culture appears to consist of imbedding the explants in a clot formed from equal parts of cockerel plasma and extract made from 7-day chick embryos. Approximately one hour later a fluid phase is added. This consists of 50% ascitic fluid or human umbilical cord serum, 40% Gey's solution, and 10% embryonic extract. Penicillin is added in a quantity sufficient to make a final concentration of 1000 units per ml. of the nutrient fluid. Carrel flasks have been found especially useful for photomicrography, but excellent results have also been obtained in roller tube cultures.

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*From the Department of Neuro-psychiatry and the Tissue Culture Laboratory, The University of Texas, Medical Branch, Galveston, Texas. Aided by a grant from the United States Public Health Service RG-333-C2. Received for publication December 20, 1949.

Outgrowth is generally observed after 4 or 5 days incubation at 37.5°C. Among the earliest migrating elements are fibers showing angular bends and beadings closely resembling well known and characteristic neurites observed in outgrowths of embryonic nervous tissue. Some of these fibers attain a length of approximately 1.0 mm. In one preparation the beadings appeared as small buds on either side of the axis of the filament producing a pattern reminiscent of dendritic gemmules. Ganglion cells resembling those described by Murray and Stout (1947) in tissue cultures of human sympathetic ganglia are occasionally present in Bodian preparations. These are seen to consist of large cells with a single prominent nucleolus, and coarsely granular cytoplasm. Some of the elements have multipolar branching processes, while others are characterized by bipolar arrangements with 5 or 6 processes at one end and with a single process in the opposite direction.

Oligodendrocytes and stellate cells believed to represent glial elements showing various modulations, are found in most cultures. Macrophages occasionally are very numerous, and types of cells believed to be related to microglia are regularly observed.

In some preparations large sheets of cells showing a high rate of mitotic activity have been interpreted as derived from the endothelium of vessels from the cortex or from pial fragments.

Work in progress on human and animal material includes studies on improving the composition of the nutrients in the medium, and the application of histochemical methods in the identification of cell types.

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Murray M. and Stout, A. T. *Am. J. of Anat.*, 1947, 80:225.

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# CALLING ATTENTION TO

DECEMBER, 1949

OF GENERAL INTEREST: E. N. Andrade reviews history of presentation of scientific information (*Proc. Roy. Soc. B.* 136/317/49). J. Dewey looks back over his 90 years to describe the philosopher-in-the-making (*Sat. Rev. Lit.*, Oct. 22, 49). N. A. Gillespie offers fine tribute to anesthetist R. M. Waters (*Brit. J. Anesth.*, 21/197/49). O. Klineberg discusses etiology and management of international tensions (*Lancet*, 2/851/Nov. 5/49). W. M. Maugham collects *A Writer's Notebook* (Doubleday, N.Y., 49, 367 pp., $4). B. Russell dilates on *Authority and the Individual* (Simon-Schuster, N.Y., 49, 80 pp., $2). R. W. Sellars & Co., edit *Philosophy for the Future: A Reformulation of Materialism* (Macmillan, Lond., 49, 610 pp., 37s6d). P. P. Wiener surveys *Evolution and the Founders of Pragmatism* (Harvard, Cambridge, 49, 288 pp., $5).

FUNDAMENTAL MATERIAL: L. J. Akkeringa describes nervous system of cardiac Purkinje fibers (*Acta. Neerland Morph.*, 6/289/49). N. Antoni and E. Lindgren confirm N. Stensen's (1638–1686) except that abdominal aortic compression causes lower limb paralysis (*Acta. Chirurg. Scand.*, 98/230/49). W. Bargmann discusses neurosecretory relations of hypothalamus and hypophysis (*Klin. Wchnschr.*, 27/617/Oct. 1/49). G. Causey and E. Palmer say failure of nerve conduction after local pressure is due to local anoxia (*J. Physiol.*, 109/220/49). C. J. Clemedson studies air blast injuries (*Acta. Physiol. Scand.*, 18/Suppl. 61/200 pp., 49). R. Collin and F. Stutinsky analyse structure and functio nof neurophypophysis (*J. Physiologie*, 41/7/49). D. B. Cowie & Co. say diffusion not filtration is chief process in fluid and electrolyte exchange across capillary walls (*Am. J. Physiol.*, 158/231/49). P. and R. Daudel discuss carcinogen-cell reactions (*Bull. Soc. Chim. Biol.*, 31/353/49). D. A. Denton considers renal regulation of extracellular fluid with regard to chloride excretion under stress (*Med. J. Austral.*, 2/521/Oct. 8/49). S. DiRienzo shows bronchial caliber increased in inspiration and start of cough with swift peristalsis from bronchi to trachea (*Radiology*, 53/168/49). O. Eranka and A. Vartiainen note pathology of drowning (*Ann. Med. Exp. Biol. Fenniae*, 27/110/49). Faraday Soc. issues symposium on crystal growth (*Disc. Farad. Soc.*, No. 5, 49, Lond., 366 pp.). P. Franceschini questions peritoneal epithelium (*Arch. Ital. Anat. Embry.*, 53/325/49). M. George and K. M. Pandalai find Mg-ribo-nucleate antagonizes penicillin action on Gram neg. bacteria (*Ind. J. Med. Res.*, 37/151/49). B. N. Halpern shows antihistaminics influence capillary permeability (*Presse Med.*, No. 65/949/Oct. 22/49). R. P. Harpin and J. H. Quastel describe relations between acetylcholine synthesis and carbohydrate and glucosamine metabolism in central nervous system (*Nature*, 164/779/Nov. 5/49). W. B. Hawkins & Co. show bile acid production from proteins and cholesterol (*J. Expt. Med.*, 90/461/49).

F. Heckner discusses plasma cells in relation to blood proteins (*Deutsch. Arch. Klin. Med.*, 194/434/49). E. Heinz and H. Netter note factors regulating colloid pressures in blood (*Biochem. Ztschr.*, 319/529/49). K. Hemmingsen finds bloodfactor *P* in 80% of people (*Acta. Path. Microbiol. Scand.*, 26/639/49). A. V. Hill compares abrupt muscle shift from rest to action to crystallization of super-cooled liquid (*Proc. Roy. Soc. B.*, 136/399, 420/49), and suggests relaxation is active process. A. Kristenson finds specific skin organ giving burning sensation which is stimulated by jellyfish toxin (*Acta. Physiol. Scand.*, 18/151/49). R. Lemberg and J. W. Legge review *Hematin Cmpds. and Bile Pigments* (Interscience, N.Y., 49, 776 pp., $15). W. V. Mayneord & Co. find high energy radiations produce radiactivity in animal tissue (*Nature*, 164/728/Oct. 29/49). E. L. McCawley shows curare stimulates CNS and in large doses gravely depresses it (*J. Pharmacol.*, 97/129/49). K. Motokawa and K. Iwama suggest electric excitability of eye as index of oxygen deficiency (*Tohuku. J. Exp. Med.*, 50/319/49). J. Paniiel discusses significance of Gram stain reaction (*Bull. Soc. Chim. Biol.*, 31/510/49). B. D. Pullingen suggests mammary tumors produce intracellular estrogen, "mammogen" (*Lancet*, 2/823/Nov. 5/49). A. and B. Pullman discuss electronic densities in steroid molecules in relation to carcinogenesis (*Bull. Soc. Chim. Biol.*, 31/343/49). K. H. Riedel reviews function of pyramidal tracts (*Schw. Med. Wchnschr.*, 79/1032/Oct. 29/49). F. J. W. Roughton and J. C. Kendrew edit volume on *Hemoglobin* in memory of J. Barcroft (Butterworth, London, 49, 329 pp., 40s). S. Seidenberg & Co. report powerful quaternary ammonium detergent disinfectant for skin, "Bradosol-Ciba" (*Schw. Med. Wchnschr.*, 79/978, 986/Oct. 15/49). R. D. Seneviratue shows sensitiveness of liver circulation to respiration, trauma, bleeding, heat and cold (*Quart. J. Exp. Physiol.*, 35/77/49). G. N. Smith and C. S. Worrell show chloramphenicol doesn't block ordinary enzymes in antibiosis (*Arch. Biochem.*, 23/341/49). R. O. Smith notes contractility of lymphatics as factor in transport of lymph (*J. Exp. Med.*, 90/497/49). E. Voetmann analyses structure of choroid plexus confirming cerebrospinal fluid formed by secretion (*Acta. Anat.*, 8/Suppl., 10/49/116 pp). E. Werle and G. Weicken find histamine in nerve fibrils but not in brain tissue (*Biochem. Ztschr.*, 319/457/49).

### Critical New Year, 1950

Worthy Popular Front: A. Rottino & Co. find 400 mgm. adenosine-5-monophosphate daily by mouth useful in relieving severe itching (*N.Y. Times*, Dec. 17/49, p. 13). R. W. Riis analyses, with bibliography, possible harm of excessive cigarette smoking (*Reader's Digest*, 29/1/50). M. Meyer is unrestrained on M. Fishbein and AMA (*Harper's*, Nov. and Dec./49).

Catching up Clinically: C. D. S. Agassiz & Co. offer methods for early diagnosis of cerebral palsy (*Lancet*, 2/1030/Dec. 3/49). H. C. Bazett reviews control of blood temperature (*Am. J. Med. Sci.*, 218/483/49). H. Benard & Co. note orthochromatic anemia improves with methionine (*Presse Med.*, Oct. 8/49, p. 885). C. H. Best & Co. describe choline pro-

phylaxis of liver damage from alcohol or sugar (*Brit. Med. J.*, 2/1001/Nov. 5/49). C. C. Burt & Co. find bisoxycoumarinyl ethyl actetate prompt controllable safe therapeutic anticoagulant (*Brit. Med. J.*, 2/1250/Dec. 3/49). F. R. C. Casson discusses psychologic regression in illness (*Lancet*, 2/681/Oct. 15/49). L. M. Davidoff and E. H. Feiring revive B. Hutchinson's ferrous carbonate treatment of trigeminal neuralgia (*Acta. Psych. Neurol.*, 24/403/49). C. E. Dent and G. A. Rose suggest multiple myelomatosis is virus disease (*Biochem. J.*, 44/610/49). T. Dougray says antihistaminics help in nausea of pregnancy (*Brit. Med. J.*, 2/1081/Nov. 12/49). J. B. Duguid notes thrombosis as factor in atherosclerosis (*Lancet*, 2/925/Nov. 19/49). Our G. W. N. Eggers, T. O. Shindler and C. M. Pomerat demonstrate contact-compression factor in healing fractures (*J. Bone and Jt. Surg.*, 31/693/49). N. H. Fairley reviews chemotherapy of malaria (*Brit. Med. J.*, 2/891/Oct. 22/49). R. R. Grunert and P. H. Phillips find low sodium and resulting low glutathione favor diabetes (*J. Biol. Chem.*, 181/821/49). F. Hansen and K. O. Moller note manifestations of arsenical poisoning (*Acta. Pharmacol. Tox.*, 5/135/49). H. V. Ingham describes interdependent inferiority and superiority feelings (*Calif. Med.*, 71/337/49). L. O. Jacobson & Co. show protective action of spleen in radiation injury (*Proc. Soc. Exp. Biol. Med.*, 70/740/49; *J. Lab. Clin. Med.*, 34/1538/49). C. Levaditi and G. Domagk review chemotherapy of tuberculosis (*Schweiz, Ztschr. Path. Bact.*, 12/557, 575/49). E. Lewin and E. Watson find 215 mgm. desoxycorticosterone acetate IM and 500 mgm. ascorbic acid IV helps in rheumatoid arthritis (*Lancet*, 2/993/Nov. 26/49). C. S. Livingood and D. M. Pillsbury note food sensitivity as factor in eczematous dermatitis (*Arch. Dermat. Syph.*, 60/1090/49). F. W. McKee & Co. describe ascites from constricted inferior vena cava (*Surg. Gyn. Obs.* 89/529/49). O. Orias describes genesis of heart sounds (*New Eng. J. Med.*, 241/763/Nov. 17/49). J. W. Papex and J. F. Bateman find ribonucleic inclusion bodies in cerebral cells of dementia praecox patients (*J. Nerv. Ment. Dis.*, 110/425/49). J. R. Penny and B. M. Balfour show ascorbate needed for mucopolysaccharide formation in wound healing (*J. Path. Bact.*, 61/171/49). S. K. Quo finds threshold stimulus of knee jerk increases with mental fatigue (*Chinese Med. J.*, 67/377/49). D. S. Russell issues *Observations on Pathology of Hydrocephalus* (*Med. Res. Counc. Sp. Rep.*, 265, Lond., 49, 102 pp., $1.4). E. Rustung shows variations in seric iron in skin diseases (*Acta. Dermat.-Ven.*, 29/Suppl. 21/140 pp./49). J. A. Salva and M. Badell find phenergan useful in radiation sickness (*Presse Med.*, 63/888/Oct. 8/49). L. A. Scheele reviews cancer research (*Bull. N. Y. Acad. Med.*, 25/671/49). G. Schwobel finds anguish a psychosomatic factor in asthma (*Arzt. Forsch.*, 3/475/Oct. 10/49). H. Selye discusses pathogenic effect of adrenal cortex in arthritis (*Brit. Med. J.*, 2/1129/Nov. 19/49), and analyses *Stress* (*Acta. Endocrin, Montreal*, 49, 800 pp., $14). J. H. Sheldon suggests hypothalamic influence in maternal obesity (*Lancet*, 2/869/Nov. 12/49). F. W. Sunderman and F. Boerner present *Normal Values in Clinical Medicine* (Saunders, Phila., 49, 845 pp., $14). J. C.

Tinsley & Co. show high oxygene tensions depress erythropoiesis (*J. Clin. Invest.*, 28/1544/49). J. Tomcsik reviews BCG (*Schweiz. Med. Wchnschr.*, 79/1071/Nov. 12/49). V. Traina notes antihemolytic effects of ascorbic acid (*Nature*, 164/843/Nov. 12/49). M. Tubangin and B. D. Cabrera recommend prolonged hetrazan treatment in filariasis (*Acta. Med. Philippina*, 6/1/49). A. Vancura notes allergic etiology in post-infection nephritis (*Acta. Med. Scand.*, 134/378/49). E. Varga & Co. observe cortin-like action of strophanthin (*Ach. Exp. Path. Pharmakol.*, 207/29/49). H. C. Weston discusses *Sight, Light and Efficiency* (Lewis, Lond., 49, 420 pp., 42s).

More Fundamental: N. J. Berridge crystallizes antibiotic nicin, blood stable polypeptide (*Biochem. J.*, 45/486/49). J. F. Danielli says long chain aldehydes are intermediate metabolites for fatty acids (*Quart. J. Micro. Sci.*, 90/309/49). M. Dixon describes *Multi-Enzyme Systems* (Cambridge Univ. Press, 49, 102 pp., 7s6d). N. M. Hancox discusses osteoclasts (*Biol. Rev.*, 24/448/49). A. Loveless and S. Revell review mode of action of mitotic inhibitors and nuclear rearrangers (*Nature*, 164/938/Dec. 3/49). M. Ostow bravely considers entropy changes in mental activity (*J. Nerv. Ment. Dis.*, 110/502/49). *Symposia:* Radiactive (why not?) Isotopes (*J. Clin. Invest.*, 28/1247–1385/49); Vitamin E (*Ann. N.Y. Acad. Med.*, 52/63-428/49). D. B. Tyler and P. Bard review motion sickness (*Physiol. Rev.*, 29/311/49).

February, 1950

Businessical: E. C. Berkeley physiologizes *Giant Brains or Machines That Think* (Wiley, N.Y., 50, 270 pp., $4). G. U. Claeton suggests *Making Work Human* (Antioch College Press, 50, 326 pp., $3.75). D. O. Hebb proposes neurophysiological theory on *Organization of Behavior* (Wiley, N.Y., 49, 335 pp., $4). Most worthy is D. E. Lilienthal's *This I Do Believe* (Harpers, N.Y., 49, 230 pp., $2.50). Look for J. McDonald's *Strategy of Poker Business and War* (Macmillan, N.Y., 50). Note Summary of recent books on economics, finance, labor relations, and management and personnel problems in *Sat. Rev. Lit.*, Jan. 28/50. C. M. Wilson crusades in *One Half the People: Doctors and the Crisis in World Health* (Sloane, N.Y., 50, 315 pp., $4).

Pharmacological: L. D. Abbott and G. W. James show vitamin $B_{12}$ affects tyrosine metabolism in remitting pernicious anemia (*J. Lab. Clin. Med.*, 35/35/50). W. Cutting & Co. show varying relation of blood concentration and metabolism of ethanol (*J. Physiol.*, 110/18/50). M. Dumont says pantothenate aids in reducing muscular cramps in pregnancy (*Presse Med.*, 58/3/Jan. 7/50). W. O. Fenn and R. Gerschman show nerves lose potassium in anoxia (*J. Gen. Physiol.*, 33/195/50). A. C. Finlay & Co. report wide activity and low toxicity of new antibiotic terramycin (*Science*, 111/85/Jan. 27/50). H. Florey & Co. offer detailed survey of *Antibiotics* (Oxford Press, N.Y., 50, 2 Vols., 1790 pp., $29.75). P. B. Hoffman and D. E. Edwards claim anhydrohydroxy-progesterone and vitamin K control

erythroblastosis in Rh neg. mothers (*Am. J. Obs. Gyn.*, 59/207/50). R. G. McCarthy and E. M. Douglas discuss *Alcohol and Social Responsibility* (Crowell, N.Y., 49, 314 pp., $3.50). D. R. Neuhaus & Co. show triodoacetytubules (*J. Lab. Clin. Med.*, 35/43/50). O. Orias & Co. show common antilamino benzoate is safe reliable pyelographic medium, excreted by kidney tubules (*J. Lab. Clin. Med.*, 35/43/50). O. Orias & Co. show common antihistaminics have consistent cardio-respiratory depressant effects (*J. Pharmacol.*, 97/492/49). R. Pratt and J. Dufrenoy discuss *Antibiotics* (Lippincott, Phila., 50, 255 pp., $5). G. Rake edits symposium on chemotherapy of tuberculosis (*Ann. N.Y. Acad. Sci.*, 52/625–788/Dec. 14/49). W. A. Sexton analyses *Chemical Constitution and Biological Activity* (Spon., Lond., 49, 434 pp., 56s). P. K. Smith reviews pharmacology of salicylates (*J. Pharmacol.*, 97, Pt. 2, or *Pharmacol. Rev.*, 1/353/49). J. E. P. Toman and J. P. Davis survey effects of drugs on electrical activity of brain (*Ibid.*, p. 425). A. Wollenberger discusses metabolic action of cardiac glycosides (*Ibid.*, p. 311).

CLINICAL: F. Buschke & Co. discuss *Supervoltage Roentgentherapy* (Thomas, Springfield, Ill., 50, 320 pp., $10.50). A. Castellani describes new hemorrhagic ulcerative disease caused by Malleomyces hemorrulogenes (*An. Inst. Med. Trop.*, 5/87/49). A. F. Coca edits symposium on allergy (*Ann. N.Y. Acad. Sci.*, 50/679–814? Dec. 26/49). G. Egrejas and P. Polonio indicate sensitivity of modified Riebeling's test of cerebrospinal fluid with colargol (*An. Portuges Psiqui.*, 1/45/49). F. Ellis and B. A. Stoll show irradiation may induce herpes (*Brit. Med. J.*, 2/1323/Dec. 10/49). A. E. Gessler & Co. continue observations on submicroscopic bodies in cancer (*Exp. Med. Surg.*, 7/237/49). O. Glasser edits 2nd Vol. *Medical Physics* (Yearbook, Chicago, 50, 1350 pp., $25). F. R. Hanson edits symposium on Combat Psychiatry (*Bull. U.S. Army Med. Dept.*, 9/Suppl. No./216 pp./49). H. Hartridge and R. Weale indicate physiological factor in flickering of stars and distant lights (*Nature*, 164/999/Dec. 10/49). J. Huxley contrasts *Heredity East and West: Lysenko and World Science*, (Schuman, N.Y., 50, 225 pp., $3). R. M. Marcusson and H. G. Wolff analyse vascular circulation in migraine (*Arch. Neurol. Psych.*, 63/42/50). W. S. McCann reviews circulatory function of respiration (*Am. J. Med.*, 8/62/50). A. Monsaingeon & Co. show impairment of intrarenal circulation following burns (*Presse Med.*, 57/1221/Dec. 25/49). L. M. Morrison and K. D. Johnson find hypercholesterolemia in coronary thrombosis (*Am. Heart J.*, 39/31/50). A. Rehak finds urethane, like nitrogen mustards, are temporarily useful in mycosis fungoides (*Dermatol.*, 98/163/49). R. Southby suggests virus etiology for pink disease (*Med. J. Austral.*, 2/801/Dec., 3/49). T. S. Szasz and A. M. Robertson propose hyperactivity of scalp muscles in producing anoxia in scalp tissue as factor in baldness (*Arch. Derm. Syph.*, 61/34/50). M. M. Tayanc says Sternberg cells in lymphogranulomatosis come from reticulum (*Turk. Tip. Cem. Mec.*, 93/189/49). L. Welch and D. Diethelm discuss adverse effect of anxiety on inductive reasoning (*Arch. Neurol. Psych.*, 63/87/50.